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SLUDGE CONDITIONING USING SO2 AND LOW PRESSURE FOR PRODUCTION OF ORGANIC FEED CONCENTRATE

Myron S. Weinberg, et al

Foster D. Snell, Incorporated

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by

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Contract #14-12-813
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Activated sludge from four municipal sewage plants was treated with 0-5% SO_2 at pressures of 50-100 psig. The filterability of the treated sludges was improved and the treatment solubilized from 26 to 44% of the initial insoluble solids. The clear, light yellow filtrates were concentrated and then spray-dried to flowable, hygroscopic, tan powders having nutritional value as an animal feed supplement. Capital investment and manufacturing costs for a plant producing 900 tons per year of a 50% concentrate or 450 tons per year of dry product was estimated at \$223,000 and \$273,000 with manufacturing costs of 6.1¢ and 13.2¢ per pound respectively.

Effects on reproductive performance of this organic feed concentrate (OFC) were investigated in rats, rabbits and chickens using nutritionally balanced diets. No teratogenic effects were seen in rats or rabbits fed 5% or 10% OFC. No alterations in reproductive parameters were noted in rats fed 5% OFC. Increased food intake and body weight gains were observed (5% OFC male and female rats) during the pre-mating feeding period. At 10% OFC levels, reduced Fertility, Viability and Lactation indexes were seen in rats and egg laying performance in chickens was suppressed. (EPA)

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ABSTRACT

A study was performed to upgrade the process of preparing SO_2/low pressure treated activated sludge, to prepare Organic Feed Concentrate (OFC) for animal feeding studies, and to evaluate the OFC for safety, as a feed supplement.

Activated sludge from four municipal sewage plants was treated with SO_2 at concentrations of 0-5% and at pressures of 50-100 psig. The filtrability of the treated sludges was improved by a factor of 6 to 40 and the treatment solubilized from 26 to 44% of the initial, insoluble solids. A clear, light yellow filtrate was concentrated and then spray-dried to a flowable, hygroscopic powder having nutritional value as an animal feed supplement. Capital investment and manufacturing costs for a plant to produce 900 tons per year of 50% concentrate or 450 tons per year of dry product was estimated from these pilot plant studies at $223,000 and $273,000 with manufacturing costs of 6.1¢ and 13.2¢ per pound respectively.

Limited pilot plant studies in which mixed sludge (about 90% primary sludge and 10% activated sludge) was treated under similar conditions showed filterability improvement and solubilization of initial insoluble solids, but to a lesser extent. Nutritional and reproduction studies were not conducted on the soluble material.

The effects of ingesting organic feed concentrate (OFC) derived from SO_2-treated activated sewage sludge on reproductive performance were investigated in rats, rabbits and chickens. Using nutritionally balanced diets OFC was fed to rats in three separate experiments; before, during and after mating; during gestation; and during gestation and lactation. Rabbits were fed during gestation only. Laying hens were fed 5, 10, 15 and 20% OFC in synthetic and commercial diets for six weeks.

No teratogenic effects were seen in rats or rabbits fed at 5 or 10% OFC. No alteration in reproductive parameters were noted in rats fed 5% OFC. Increased food intake and body weight gains were recorded (5% OFC male and female rats) during the premating feeding period. Reduced Fertility, Viability and Lactation indexes were seen at 10% OFC levels. Egg laying performance was supressed in the 5 and 10% OFC groups. Egg laying ceased entirely in birds fed 15 and 20% OFC. The OFC preparation used in this study contained very little calcium, high levels of phosphate and relatively high levels of heavy metals, which could account for the observed depression in egg laying.

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Section I

CONCLUSIONS

The project was initiated to upgrade the SO_2 /low pressure process for preparing Organic Feed Concentrate (OFC) from activated sludge, to prepare a significant amount of OFC for animal feeding studies, and to evaluate the safety of the OFC as a feed supplement through recognized toxicological procedures.

The following conclusions may be drawn:

- The technical feasibility for preparing an animal feed concentrate (OFC) from activated sludge has been demonstrated in pilot plant equipment.
- This process will also reduce the quantity of solids to be disposed, will return food value to the economy by producing a usable by-product, and may increase the throughput of existing sewage plants.
- The economic aspects of this process should not rest entirely on the actual value of the product produced, but must take into consideration other economies such as present costs of disposal, raw material value and possible increase in sewage plant efficiency.
- No teratogenic effects were seen in rats or rabbits fed OFC.
- No alterations in reproductive parameters were noted in rats fed 5% OFC, but minor changes were seen at the 10% level.
- Increased food intake and body weight gains were recorded in rats fed 5% OFC during the pre-mating period.
- Egg laying was reduced in hens fed this preparation of OFC.

The excellent growth in rats fed 5% OFC may be due to the good quality of the proteins in the material; and the minor effects observed in reproduction parameters for animals fed higher levels of OFC may be attributed to the relatively high levels of phosphate and heavy metals found in this particular preparation.

Simple procedures are available for removing potentially toxic substances found in the preparation of OFC examined in this study.

Thus, OFC appears to be safe for use as a feed supplement at a 5% level in the diet and probably at higher levels when procedures are instituted to remove phosphates and heavy metals.

Section II

RECOMMENDATIONS

The feasibility of converting activated sludge into a product having nutritional value has been demonstrated. However, to explore the full potential of this process, we suggest the process be demonstrated on a commercial size, or at least in semi-commercial size unit, as an adjunct to an existing sewage facility. This will serve several purposes:

- Realistic process and cost data can be developed
- Studies of factors not previously examined can be conducted
- Large enough quantities of OFC can be prepared to be able to conduct feeding studies on poultry and meat producing animals.

We have shown that mixed sludge can be partially solubilized by this process. Nutritional and toxicological studies should be conducted on the soluble portion. Should this fraction have food value and be non-toxic, then the scope of this invention could be broadened with the potential of eliminating the anerobic digestion stage of sewage treatment.

The insoluble portions remaining after this treatment should be further examined to determine:

- the possibility of hydrolyzing them further
- if they need be separated from the OFC
- possible utilization as fuel

The results of the present study indicate no markedly toxic materials in OFC, except possibly high levels of heavy metals or phosphorus - all of which can be removed by appropriate treatment of the activated sludge. Further, the nitrogen constituents, particularly amino acids, offer some positive features as a dietary constituent.

Thus, it is important to prepare OFC in such a manner as to eliminate heavy metals and phosphorus, and to feed this material to food animals including sheep, cattle, pigs and chickens to determine its potential as a feed constituent.

Also, since OFC will be judged as a food additive, the new OFC should be subject to

- a three-generation multigeneration study in rats, and a
- 12 months safety evaluation study in dogs and monkeys

These latter studies are recommended by the Food and Drug Administration for the safety evaluation of food additives and thus should be applied to the new OFC.

Section III

INTRODUCTION

On the basis of total volume processed, sewage treatment is the largest industry in the world. A sewage treatment facility is really a chemical processing plant with two lines:

- a waste treatment line designed to produce an effluent suitable for discharge to natural waters
- a line designed to dispose of the product of the effluent producing line which we call sludge.

Sludge is not an attractive product. It does not look good. It does not smell good. For the most part it has been treated as a waste rather than a product or a raw material. It has been dumped into the ocean, a handy river, or a man-made lagoon. It has been buried and incinerated. It is a problem because it is produced in quantity near large cities where it fills no human need and could menace health and destroy aesthetic values.

Yet sewage sludge contains various materials known to have value. Conditioning treatments have been devised and applied to sludge to transform it into useful products:

- It has been dried to produce the fertilizer Milorganite^R.
- It has been thickened, steam-injected, filtered, and dried to produce fertilizer (Porteus process).(1)
- It has been wet-air oxidized under high pressure to convert the organics to carbon dioxide and to recover the energy evolved (Zimpro process).(2)
- It has been wet-air oxidized using lower pressure.(3)

While these treatments dispose of sewage sludge in a manner that is ecologically desirable, they are not economically attractive. The cost of processing (and shipping, in the case of fertilizers) are high and the sale price of the product is not high enough to offset them. Two interesting processes have been tried recently to use sludge to reclaim and upgrade land. In one, the liquid material is pumped through pipeline to farmland well beyond the urban and sub-urban areas where it is produced. In the other, dewatered sludge is composted to produce a soil conditioner.(4) These methods are ecologically sound and may prove to be economically sound as well.

The chemical composition of sludge (polysaccharides, protein, fat, and inorganic complexes) suggests its use also as a feed. This use was reported by Hackler, Neuman, and Johnson(5) and Hurwitz(6) in 1957.

The activation of sludge is an aerobic fermentation process in which the organic matter in sewage is metabolized and assimilated as the nutrient media for the growth of microorganisms. This process is equivalent to the growth of single-celled protein. Hacker et at. reported that dried activated sludge was equal in nutritional quality, as measured by nitrogen retention in lambs, to soybean meal and urea-containing rations of similar crude protein content; but that the total nitrogen of sludge-containing ration had an apparent lower digestibility in rats (52%) and a moderately low biological value (51%). It seemed to us that there was a valid scientific rationale for the hope that activated sludge could be upgraded for regular use in animal feed.

Sulfurous Acid Solubilization of Organic Material

Acid digestion of organic material is a process used to induce chemical and physical changes in organic material. Strong mineral acids are used in some of these processes, but dilute sulfurous acid (SO_2) has been shown to be an excellent agent for the solubilization of naturally occurring substances. It provides enough acidity and yet inhibits the formation of additional products usually called humins.

A whole technology has been built on the use of sulfite for delignification of pulp for the paper industry.(7) McKinney(8) reported on the efficacy of sulfurous acid for the preparation of white soya protein extracts in 1949. Baker et al.(9) undertook a careful study of the sulfurous acid treatment of casein, taking special note of its protective effect on the tryptophan content of the humin-free extract.

It appears that the advantages of sulfurous acid are best realized in treatments of heterogeneous natural substances containing both polysaccharides and proteins. Where these substances are present together, sulfurous acid prevents or minimizes the formation of black or brown humins. For just this reason the packing house industry often uses sulfite in the wet-rendering process to digest inedible residues (hair, feathers, scrap, bone). In this process the wastes are cooked at relatively low pressure (60 lb steam) to permit recovery of industrial fat and protein for the manufacture of bacteria culture media and specialty protein foods.

In our first report(10) we detailed the results of a research program designed to show that the known technologies of the wood pulp, meat waste, and other industries could be applied to the treatment of activated sludge. Use of a 0.5% sulfurous acid and arbitrarily chosen treatment parameters of sludge at 140°C. for one hour, increased the soluble solids content of the activated sludge by 20%. Concentration of the solubilized extract produced a syrup; 85% of the solids content of the syrup was organic. By TKN estimates, over 20% of these solids were protein. An amino acid analysis indicated this syrup could be of value as a animal feed. In rat feeding studies (at a 10% level in the diets) we demonstrated an EFU (Efficiency of Food Utilization) of 42 for the organic feed concentrate as compared to 39 for cane molasses.

Having shown the utility of sulfurous acid and pressure in conditioning activated sludge and the feed efficiency of the organic molasses produced by the process, we undertook a program to

- Establish parameters (within limits) for the treatment of sludge:
  - time
  - pressure
  - SO_2 concentration
  - sludge age, source, and solids concentration
- Upgrade the process from laboratory scale to pilot plant size equipment
- Produce 150 lbs of treated dried organic feed concentrate (OFC) by spray drying the organic molasses

- Analyze the OFC for ash, nitrogen, sulfur, heavy metals, and amino acids
- Evaluate the efficiency of the OFC to support growth, mating, pregnancy and lactation in the rat and rabbit
- Evaluate the safety of the developing fetus in the rat and rabbit
- Evaluate the efficiency of the OFC to support egg laying in chickens
- Evaluate the taste of eggs produced by chickens fed OFC
- Determine the sterility, combustibility and heat value of the filter cake
- Prepare an economic and engineering analysis of the preparation of the 150 lb batch of OFC
- Determine the value of the pilot plant as a model for the design of a municipal demonstration unit.

Section IV

PROCESS ENGINEERING

LABORATORY STUDIES

The effectiveness of heating activated sludge with sulfur dioxide (SO_2) at low pressure has been demonstrated qualitatively. Results indicate that (1) the filtration rate of sludge heated with SO_2 at low pressure is higher than if the sludge was heated at low pressure alone, (2) the amount of soluble solids present in the filtrate of the treated sludge was greater in the presence of SO_2, and, (3) these soluble solids have value as an animal feed supplement.

The purpose of this program is to determine the technical economic feasibility of the process and prepare sufficient organic feed concentrate (OFC) to establish its biological safety.

Before proceeding to the pilot plant scale evaluation of the process, it was considered necessary to examine a number of parameters, in detail, on a laboratory scale. This phase of the work will:

- Establish the important parameters which maximize the yield of soluble solids and/or gives the highest filtration rate
- Screen and quantify these parameters
- Characterize activated sludge from several local sewage plants
- Select a sewage plant to use as a source of raw material for the pilot plant studies and preparation of the OFC to be subsequently used for biological examination.

Identification of the Variables

Our early work indicated that heat treatment of activated sludge at low pressure solubilized some of the insoluble solids present and increased the filterability of the sludge. Addition of SO_2 before treatment, as sulfurous acid, not only increased the filterablity further, but also increased the amount of solids solubilized.

The data given in Tables 1 and 2, taken from our early work, indicated that SO_2 concentration, treatment time and pressure are factors which effect the solubilization and filterability. That is increasing SO_2 concentration, increased pressure and longer treatment time favor increased filterability and increased solubilization.

Table 1

GRAMS OF SOLUBLE SOLIDS IN 50 ML OF TREATED LIQUOR PREPARED UNDER VARIOUS CONDITIONS

	Solids in 50 ml		
SO_2 Concentration	1 hour @ 100 psig	6 hours @ 15 psig	1 hour @ 15 psig
No SO_2 Added	-	0.51	0.21
2.5% SO_2 Added	1.47	1.41	0.71
1.2% SO_2 Added	1.38	0.96	0.48
.63% SO_2 Added	1.21	0.68	0.48
.33% SO_2 Added	1.18	0.64	0.44

Note: Total available solids in 50 ml would be 3 grams

Table 2

ABSOLUTE RATE OF DEWATERING

(200 ml sample)

Treatment	Filtrate Collected (ml)	Time to Achieve a Dry Cake (min)
A	147	420
B	150	4
C	175	2

A = untreated raw activated sludge
B = raw activated sludge autoclaved for 1 hr at 30 psig
C = raw activated sludge containing 0.5% by weight SO_2 autoclaved for 1 hr at 30 psig

It was also suspected that the composition of the activated sludge, being an end product of a fermentation process, could change on standing. Since considerable time may elapse between the beginning and end of a series or set of experiments, the age of the sludge was considered a variable.

Source of Activated Sludge

Our early work was conducted on activated sludge obtained from the Little Ferry, N.J. sewage plant. Screening for the important or significant variables was conducted on sludge obtained from the same location.

Procedure

In our earlier work, samples of sludge to be treated were introduced into pint-size, Ball-brand Mason jars sealed with rubber gaskets and wire-clamped glass lids and autoclaved in the horizontal pressure device shown in Figure 1. On releasing the pressure after treatment, it was noted that the glass jars had a tendency to crack. This was particularly true when the pressure was released rapidly.

Epoxy-lined aerosol cans were substituted for the glass jars. This had several advantages: heat transfer was better and faster, and the pressure could be released quickly and cans cooled rapidly by immersion in cold water.

About 160 g of activated sludge was introduced into the cans. Various amounts of SO_2 (as sulfurous acid) were added and the cans sealed. The cans were placed in the horizontal autoclave and (1) the steam turned on until the autoclave reached the desired pressure, (2) the pressure was maintained within ± 2 psi for the required time, (3) the pressure was released, and (4) the samples were removed and placed in cold water. When the samples reached ambient temperature, filtration rates and conversion yields were measured immediately.

Filtration rate: The filtration rate was measured by filtering 100 ml of treated or untreated sludge through a Whatman 41H analytical paper, using a Buchner Funnel maintained at 20 in vacuum (the apparatus used is shown in Figure 2). The filtration rate is a function of the time, in seconds, to collect a specified quantity of filtrate.

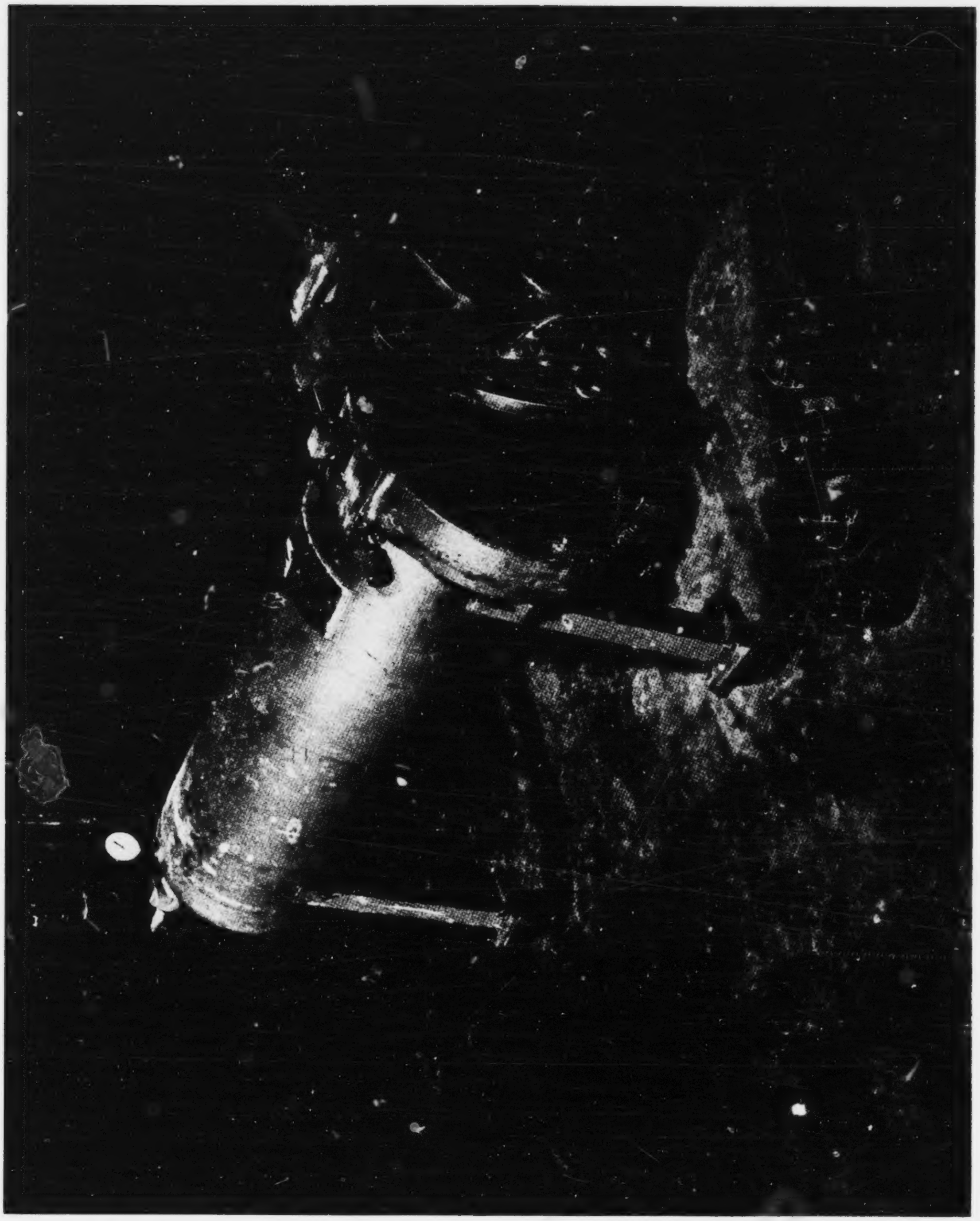


Figure 1. HORIZONTAL PRESSURE AUTOCLAVE

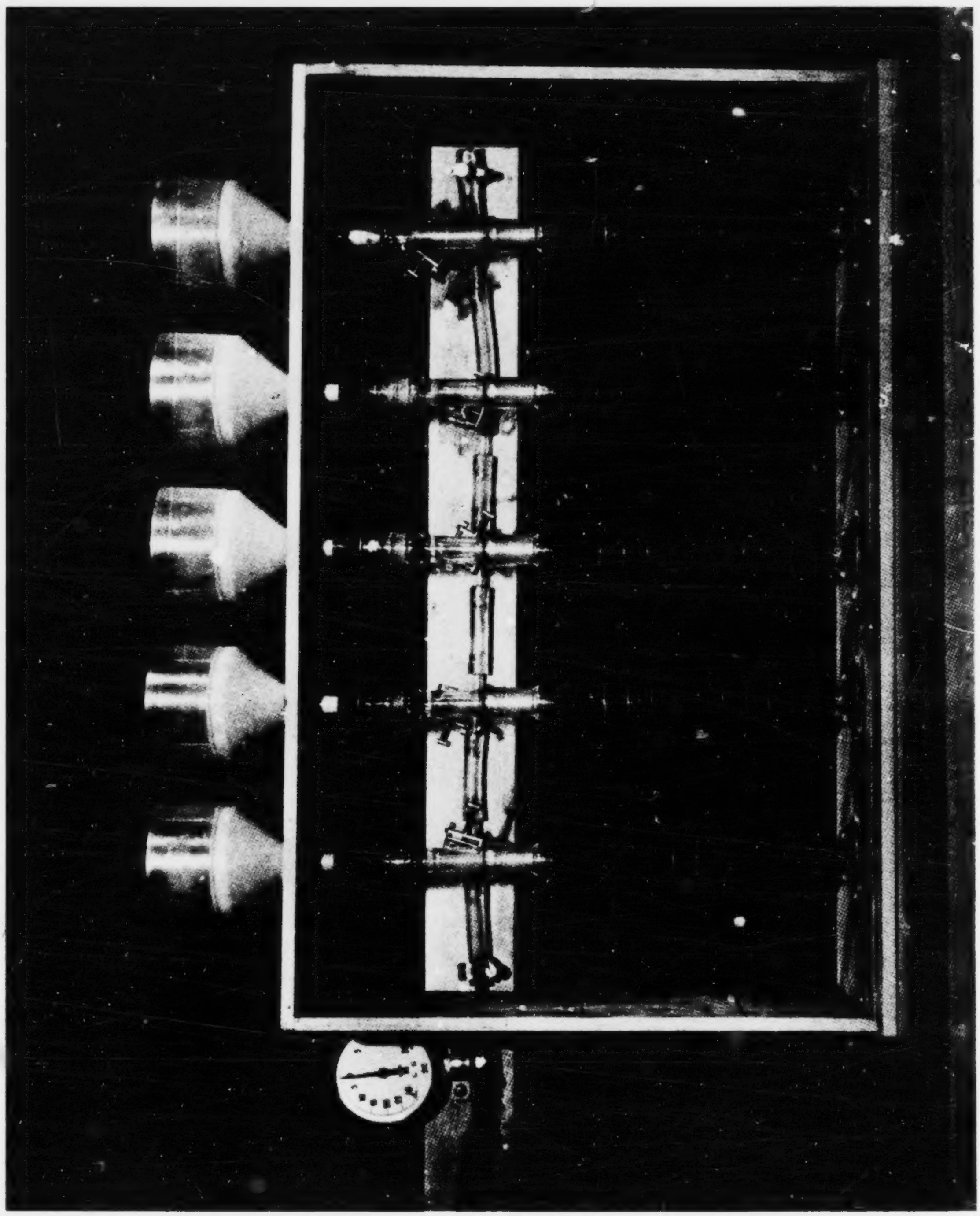


Figure 2. FILTRATION RATE APPARATUS

Conversion: Conversion is defined as the quantity of insoluble solids solubilized and expressed in percent.

It is difficult to measure the amount of solids remaining after treatment because of the difficulty in dewatering the sludge. Therefore, conversion was measured by determining the increase in the amount of soluble solids present in the water portion of the treated sludge by evaporating about 10 ml of the filtrate obtained during the filtration rate determination to dryness at 103°C, making corrections for the soluble solids present in the untreated sludge, and comparing this to the amount of insoluble solids in the untreated sludge.

$$\% \text{ Conversion} = \frac{\begin{pmatrix}\text{soluble solids}\\ \text{after treatment}\end{pmatrix} - \begin{pmatrix}\text{soluble solids}\\ \text{before treatment}\end{pmatrix}}{(\text{insoluble solids before treatment})}$$

Screening the Variables

Effect of age of the activated sludge: Fresh activated sludge was obtained from the Little Ferry, N.J. sewage plant and stored at room temperature for a period of 48 hours. Air was bubbled through the mixture throughout this time to keep it aerobic. Nonetheless, odor development was noticeable, indicating anerobic fermentation was taking place.

Subsequently, samples of fresh, 24-hour old and 48-hour old samples of activated sludge were treated with various amounts of SO_2 at 100 psig for 60 minutes. Filtration rate and conversion was measured immediately after treatment.

Characteristics of the samples before treatment are summarized in Table 3. As can be seen, filtrate COD, total solids and dissolved solids decrease rather regularly throughout the period, pH increased during the first 24 hours.

Table 3

CHARACTERISTICS OF AGED ACTIVATED SLUDGE

	Fresh	After 24 hrs	After 48 hrs
Filtrate COD (ppm)	100	80	50
pH	7.0	7.2	7.2
Total (Solids (%))	1.5	1.4	1.2
Total (Dissolved Solids (%))	0.17	0.17	0.11

Tables 4 and 5 show the conversion and filtration rates, respectively, of samples of the sludges shown in Table 3. Data shown in Table 4 are for duplicate samples, while only single samples were used in Table 5.

Table 4

CONVERSION AS A FUNCTION OF SLUDGE AGE AND SO_2 CONCENTRATION
(percent conversion)

Age of Sludge	No SO_2	SO_2 (% based on Total Solids) 1.5	3.0	4.5
Fresh	24.8	34.6	35.3	42.9
	27.0	30.8	39.9	34.6
24 hours	33.3	24.1	50.4	52.0
	24.4	23.6	39.1	34.2
48 hours	22.8	31.8	32.4	39.4
	24.8	28.3	36.6	31.8

Table 5

FILTRATION RATE AS A FUNCTION OF SLUDGE AGE AND SO_2 CONCENTRATION
(seconds required to collect 50 ml of a 100 ml sample)

Age of Sludge	No SO_2	SO_2 (% based on Total Solids) 1.5	3.0	4.5	6.0
Fresh	3000	865	120	40	23
24 hours	3062	1020	962	220	107
48 hours	6100	4500	2366	1247	316

An analysis of variance (Table 6) of the conversion data presented in Table 4 shows that the age of the sludge has little effect on conversion. While no analysis of variance was made on the filtration data, it is obvious that age has a real effect on filtration rate - treated fresh sludge filtering much faster than treated aged sludge.

Table 6

ANALYSIS OF VARIANCE FOR DATA PRESENTED IN TABLE 4

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio
A=Age of Sludge	71.320	2	35.6600	1.18
B=SO_2 concentration	818.675	3	272.8917	9.03*
A x B Interaction	204.675	6	34.1125	1.13
Error	362.485	12	30.2071	

* Significant at 1% level

The pH of activated sludge containing various amounts of SO_2 (added as sulfurous acid) was measured before and after treatment as shown in Table 7. As would be expected, after addition of SO_2, the samples became more acid; this effect being most pronounced in the fresh samples. All sludge samples became more acid during treatment--the treated fresh samples had the lowest pH.

Table 7

CHANGE IN pH AS A FUNCTION OF SLUDGE AGE AND SO_2 CONCENTRATION
(% based on total solids)

Sludge Age	0% Before Treatment	0% After Treatment	1.5% Before Treatment	1.5% After Treatment	3% Before Treatment	3% After Treatment	4.5% Before Treatment	4.5% After Treatment
Fresh	7.0	6.3	6.1	5.8	5.4	4.9	4.9	4.3
	7.0	6.2	6.1	5.8	5.2	4.4	4.9	4.4
24 hours	7.2	6.4	6.3	6.3	5.8	5.7	5.3	4.6
	7.2	6.2	6.4	5.8	5.6	5.3	5.3	4.6
48 hours	7.2	6.4	6.9	6.2	6.4	5.8	6.0	5.3
	7.2	6.4	6.9	5.8	6.4	5.8	6.0	5.2

Note: Samples were treated for 1 hr at 100 psig.

Effect of treatment time, pressure and SO_2 concentration on conversion and filtration rate: The data shown in Tables 8 and 9 are the result of varying treatment time, pressure and SO_2 concentration on conversion and filtration rate respectively. Activated sludge, obtained from Little Ferry, N.J. was treated for 30 to 60 minutes at pressures from 50 to 125 psig and with 1.5 to 4.5% SO_2 based on the total solids in untreated raw sludge.

Table 8

CONVERSION OF ACTIVATED SLUDGE
(percent)

Characteristics of Raw Activated Sludge Before Treatment:

pH	7.1 - 7.3
Total Solids (%)	1.4
Soluble Solids (%)	0.10-0.11

Levels of Time (min)	Levels of Pressure (psig)	Levels of SO_2 (% based on total solids)			
		No SO_2	1.5	3.0	4.5
30	50	16.9	17.7	18.5	20.0
45		20.0	20.8	23.1	24.6
60		20.8	23.1	26.2	24.6
30	75	24.6	28.5	26.9	34.6
45		22.3	22.3	26.9	27.7
60		24.6	24.6	29.2	31.5
30	125	26.2	30.0	33.1	36.9
45		26.2	33.1	34.6	37.7
60		26.2	33.1	34.6	37.7

Table 9

FILTRATION RATE OF TREATED ACTIVATED SLUDGE
(seconds to collect 50 ml of a 100 ml sample)

Levels of Time	Levels of Pressure	Levels of SO_2 (% based on total solids)			
		0.0	1.5	3.0	4.5
30	50	2730	1560	225	75
45		1740	853	187	61
60		1280	375	113	37
30	75	323	83	43	42
45		555	391	51	48
60		640	105	70	44
30	125	38	32	27	24
45		43	33	25	15
60		37	23	12	10

Analysis of Variance Tables for conversion and filtration rate are shown in Table 10 and 11 respectively. The data presented in Tables 8 and 9 are the result of single measurements and there is not sufficient background of information on the treatment under study to provide a reliable external estimate of error variance. The three factor interaction term was, therefore, used as an estimate of the error variance.

Table 10

ANALYSIS OF VARIANCE TABLE
(percent conversion)

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio
Time (t)	22.5588	2	11.2794	7.17*
Pressure (P)	738.1922	2	369.0961	234.54*
SO_2 concentration (C)	275.4115	3	91.8038	58.34*
t x P interaction	75.2927	4	18.8232	11.96*
t x C interaction	9.4837	6	1.5806	1.00
P x C interaction	50.3297	6	8.3883	5.33*
t x P x C interaction	18.8845	12	1.5737	

*Significant at the 1% level

The average effect of all main factors, e.g. treatment time, pressure and SO_2 concentration are significant. That is, increasing the level of any of the factors will increase conversion. However, the significance of the time-pressure and pressure-SO_2 concentration interaction terms indicates that the effect of one factor depends upon the level of the other. Thus, at 50 psig and 125 psig increasing treatment time from 30 minutes to 45 minutes produces a large increase in conversion but only a small increase when time is increased to 60 minutes. At 75 psig increasing treatment time from 30 minutes to 45 minutes actually decreased conversion but on increasing the treatment time to 60 minutes again increases conversion but not to the level of that obtained at 30 minutes. While the pressure-concentration interaction is significant, it is not as important as the time-pressure interaction. Highest conversions are obtained at the highest levels of pressure and SO_2 concentration.

Table 11

ANALYSIS OF VARIANCE TABLE
(Filtration Rate)

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio
Time (t)	249328	2	124674	2.16
Pressure (P)	3634500	2	1817250	31.52*
SO_2 concentration (C)	3488361	3	1162787	20.17*
t x P interaction	781275	4	195319	3.39
t x C interaction	207449	6	34575	0.60
P x C interaction	3534146	6	589024	10.22*
t x P x C interaction	6917793	12	57648	

*Significant at the 1% level

Interpretation of the data in Table 9 indicates that pressure and SO_2 concentration are highly significant and that treatment time has little influence on the filterability of the treated sludge. That the pressure-concentration interaction is significant indicates filterability can be increased by either increasing the pressure and decreasing the SO_2 concentration or vice versa.

Exploration of the Response Area

It has been established that the age of the activated sludge has some influence on filtration rate, but little on conversion; treatment time, pressure and SO_2 concentration influences filtration rate.

It was decided to explore the response area of filtration rate and conversion within the ranges of interest. These characteristics should be related to the three independent variables such that $Y=f(x_1, x_2, x_3)$ where x_1 is the pressure in psig ranging from 60 to 125 psig; x_2 is the treatment time in minutes ranging from 15 to 60 minutes, and; x_3 is the SO_2 concentration in percent (based on the total solids of the untreated activated sludge). For ease of calculation these variables were coded as follows:

	Factor Levels		
Coded Levels	x_1	x_2	x_3
1.215	125	60	2.5
1.0	119	56	2.28
0	92.5	37.5	1.25
-1.0	66	19	0.22
-1.215	60	15	0

Thus, denoting the variables measured in units of the experimental design by x_1, x_2, and x_3 respectively, then

$$x_1= \frac{(\text{psig})-92.5}{26.5}$$

$$x_2= \frac{(\text{time})-37.5}{18.5}$$

$$x_3= \frac{(\text{concentration})-1.25}{1.03}$$

Treatment consisted of reacting activated sludge from Little Ferry, New Jersey in sealed aerosol cans as previously described. The trials were run in random order at the levels indicated in Table 12. The filtration rates and conversions obtained are listed.

Table 12

THE EXPERIMENTAL DESIGN USED AND THE FILTRATION RATES AND CONVERSIONS OBTAINED

Trial	Factor Level			Filtration Rate(1) (seconds)		% Conversion	
	x_1 (psig)	x_2 (min)	x_3 (%)	Obs.	Calc.	Obs.	Calc.
1	-1	-1	-1	532	396	19.3	19.8
2	+1	-1	-1	68	92	30.6	27.0
3	-1	+1	-1	210	245	22.6	23.0
4	+1	+1	-1	276	567	22.5	30.5
5	-1	-1	+1	90	92	20.1	21.6
6	+1	-1	+1	26	21	27.0	31.2
7	-1	+1	+1	62	57	26.5	27.1
8	+1	+1	+1	14	10	36.8	35.2
9	-1.215	0	0	406	175	21.8	22.5
10	+1.215	0	0	23	28	32.4	31.7
11	0	-1.215	0	189	97	23.4	25.0
12	0	+1.215	0	33	54	29.6	29.2
13	0	0	-1.215	57	18	27.7	24.6
14	0	0	+1.215	24	30	33.8	29.6
15	0	0	0	40	72	26.5	27.1
16	0	0	0	44	72	29.0	27.1
17	0	0	0	67	72	30.8	27.1

(1) Time to collect 90 ml of filtrate from a 100 ml sample.

Regression Analysis Tables for filtration rate (Table 13) and conversion (Table 14) indicates that the time factor (within the ranges explored) is not significant in either conversion or filtration. Pressure and SO_2 concentration are significant. When an overall test of this kind has confirmed the adequate fit of an equation of a particular degree, the fact that an individual coefficient is or is not significantly different from zero has no particular relevance. That coefficient should still be included in the "Equation of Best Fit".

The regression analysis indicates that first order equations for filtration rates and conversion are adequate to describe the response areas in the region of the design.

The equation for filtration rates (in coded units) is:

$$\log Y = 1.8589 - 0.3173x_1 - 0.1046x_2 - 0.3168x_3$$

The equation for conversion (in coded units) is:

$$Y = 27.08 + 3.77x_1 + 1.73x_2 + 2.08x_3$$

These equations indicate that filterability and conversion can be improved by increasing the levels of all three factors with treatment time being a minor factor. Using these equations we calculated the responses listed in Table 12.

Table 13

REGRESSION ANALYSIS TABLE (FILTRATION RATE)

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio
Total sum of squares	62.2239	17		
b_0	58.7468	1	58.7468	
b_1	1.1026	1	1.1026	77.64*
b_2	0.1201	1	0.1201	8.46
b_3	1.0995	1	1.0995	77.43*
Residual	1.1549	13		
Lack of fit	1.1265	11	0.1024	7.21
Error	0.0284	2	0.0142	

*Significant at 5% level

Table 14

REGRESSION ANALYSIS TABLE (CONVERSION)

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio
Total sum of squares	12865.7	17		
b_0	12468.7139	1	12468.7139	
b_1	155.5777	1	155.5777	66.72*
b_2	32.7285	1	32.7285	14.04
b_3	47.5111	1	47.5111	20.38*
Residual	161.1688	13	12.3976	
Lack of fit	156.5055	11	14.2278	6.10
Error	4.6633	2	2.3317	

*Significant at 5% level

5% significance level (11,2) = 19.40
5% significance level (1, 2) = 18.51

CHARACTERIZATION OF LOCAL ACTIVATED SLUDGE PLANTS

Four local municipal activated sludge plants were characterized--Madison, New Jersey, Parsippany-Troy Hills, New Jersey, Livingston, New Jersey and Morristown, New Jersey. The purpose for these characterizations was to assist us in choosing the plant which would supply the raw activated sludge to be used in preparing the OFC for biological testing.

It appears that all of the plants sampled have similar characteristics. The normal variations in total solids were noticed, and the degree of fermentation is similar. It was therefore decided, that since the Parsippany plant was only a few miles from the pilot plant location and it was our understanding that this plant receives only municipal sewage (no industrial wastes are present), to use activated sludge from this location.

Results of our analysis of the four local plants are summarized in Table 15.

Table 15

CHARACTERISTICS OF MUNICIPAL ACTIVATED SLUDGE PLANTS

Sample Rec'd		Total Sample				Filtrate				
Date	Time	pH	% Total Solids	% Ash	% Volatiles	% Dissolved Solids	COD PPM	Ammonia (N) PPM	Nitrate (N) PPM	Nitrite PPM
					Madison, New Jersey					
6/10/70	8:50 am	7.0	1.0	0.2	0.8	0.07	100	16	0.15	0.035
6/10/70	12:15 pm	6.9	0.9	0.2	0.7	0.08	100	18	0.15	0.075
6/11/70	8:50 am	7.1	0.7	0.2	0.5	0.07	150	18	0.25	0.015
6/12/70	8:55 am	6.9	1.0	0.3	0.7	0.07	120	20	0.15	0.020
6/12/70	11:55 am	6.7	0.9	0.3	0.6	0.09	90	28	0.20	0.015
6/15/70	9:10 am	7.2	0.8	0.2	0.6	0.01	100	24	0.35	0.030
6/15/70	11:15 am	7.0	1.0	0.3	0.7	0.10	140	24	0.30	0.035
6/16/70	8:50 am	7.2	0.9	0.3	0.6	0.07	100	22	0.30	0.020
6/17/70	9:30 am	7.1	0.8	0.2	0.6	0.06	120	22	0.30	0.015
					Morristown, New Jersey					
6/10/70	8:50 am	6.85	0.9	0.2	0.7	0.06	160	8	0.15	0.040
6/10/70	12:20 pm	7.1	0.8	0.2	0.6	0.05	120	12	0.15	0.025
6/11/70	8:50 am	7.0	1.0	0.3	0.7	0.05	110	14	0.30	0.020
6/12/70	8:40 am	6.7	1.2	0.4	0.8	0.04	100	16	1.00	0.020
6/12/70	11:40 am	6.8	1.1	0.3	0.8	0.06	120	20	0.30	0.015
6/15/70	8:40 am	7.0	0.7	0.2	0.5	0.09	80	18	0.30	0.025
6/15/70	11:45 am	7.0	0.7	0.2	0.5	0.06	90	24	0.20	0.030
6/16/70	8:40 am	7.1	0.7	0.2	0.5	0.04	70	12	0.30	0.015
6/17/70	8:40 am	7.0	0.7	0.2	0.5	0.04	70	10	0.30	0.010

Table 15 Con't.

CHARACTERISTICS OF MUNICIPAL ACTIVATED SLUDGE PLANTS

Sample Rec'd		Total Sample				Filtrate				
Date	Time	pH	% Total Solids	% Ash	% Volatiles	% Dissolved Solids	COD PPM	Ammonia (N) PPM	Nitrate (N) PPM	Nitrite PPM
				Livingston, New Jersey						
6/18/70	1:30 pm	7.4	0.5	0.1	0.4	0.10	90	30	0.30	0.030
6/19/70	8:45 am	7.0	0.4	0.1	0.3	0.08	0	10	2.50	1.500
6/19/70	1:20 pm	7.3	0.4	0.1	0.3	0.09	90	12	0.400	0.400
6/22/70	8:50 am	7.2	0.6	0.2	0.4	0.09	50	14	0.300	1.500
6/22/70	1:05 pm	7.4	0.5	0.1	0.4	0.10	90	16	0.400	0.040
6/23/70	8:50 am	7.2	0.4	0.1	0.3	0.08	50	10	2.000	2.000
6/23/70	12:50 pm	7.3	0.5	0.1	0.4	0.10	60	20	0.300	0.002
				Parsippany-Troy Hills, New Jersey						
6/18/70	10:00 am	7.2	0.6	0.2	0.4	0.07	100	20	0.200	0.020
6/19/70	9:00 am	7.1	0.5	0.2	0.3	0.06	80	24	0.300	0.008
6/19/70	1:30 pm	7.1	0.5	0.2	0.3	0.06	140	28	0.300	0.030
6/22/70	10:00 am	7.1	0.6	0.2	0.4	0.07	140	24	0.250	0.020
6/22/70	1:25 pm	7.2	0.6	0.2	0.4	0.10	80	28	0.300	0.008
6/23/70	9:05 am	7.2	0.6	0.2	0.4	0.06	80	22	0.300	0.075
6/23/70	1:10 pm	7.2	0.9	0.3	0.6	0.07	100	24	0.400	0.006

PILOT PLANT STUDIES - SMALL SCALE

Activated Sludge

Procedures

All treatments were conducted in a 5-gallon reactor equipped with a stirrer rotating at approximately 180 rpm. The reactor was jacketed and contained a heating coil. The reactor was heated and cooled by feeding steam or cold water respectively to both jacket and coil simultaneously.

The reactor was charged with 4 gallons of waste water sludge, the stirrer started and steam fed to both coil and jacket in order to heat the charge to the desired pressure in the shortest possible time. In this manner reaction pressures of 75 to 120 psig were reached in 10 to 12 minutes.

After treating the sludge for the desired time, heating was stopped and the charge cooled to room temperature by circulating cold water through both coil and jacket until the internal pressure reached 0 psig. The mixture was then discharged and measurements made immediately to determine soluble solids content and filtration rate.

Selection of Variables

Three variables were considered important in this process--treatment time, pressure and SO_2 concentration.

The pressure and SO_2 concentration were studied in the ranges of 50 to 100 psig and 0 to 5% SO_2 (based on total sludge solids) respectively. There were indications from laboratory scale work, that treatment of activated sludge for periods longer than 15 minutes produced little change in the quantity of soluble solids produced. Such being the case, statistical treatment of the experimental program was greatly simplified and reduced the number of trials substantially.

To verify this, several experimental trials were made in the stirred reactor, at treatment times ranging from 0 to 30 minutes at pressures of 75 psig and 1.0% and 1.5% SO_2 for 20 and 300 minutes. The results are summarized in Table 16.

Table 16

EFFECT OF TREATMENT TIME ON CONVERSION AND FILTRATION RATE

Analysis of Raw Activated Sludge Before Treatment:

Filtration Rate = 188 seconds
Total Solids = 0.9%
Soluble Solids = 0.04%

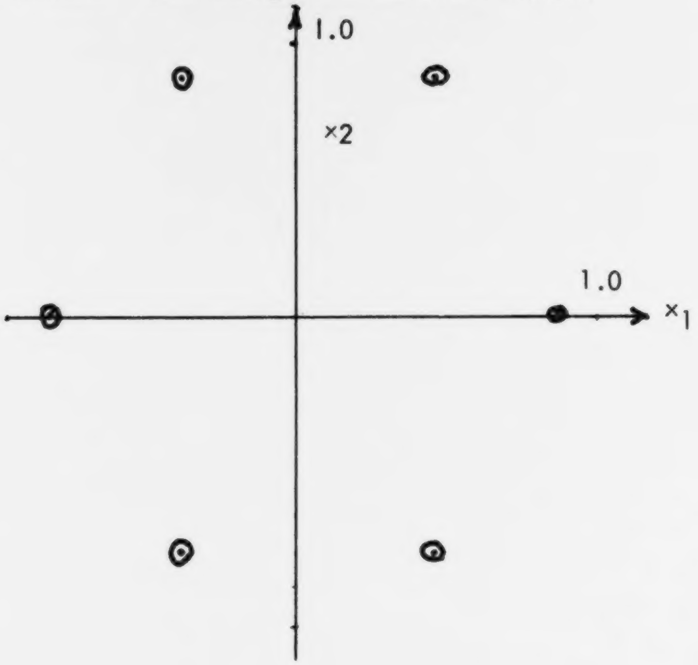
Treatment Time (minutes)	Filtration Rate (seconds)	Soluble Solids (%)	Conversion (%)
	1% SO_2 and 75 psig		
0[1]	71	0.23	22.1
10	8	0.26	25.6
20	7	0.26	25.6
30	6	0.26	25.6
	1.5% SO_2 and 125 psig		
20	2.5	-	38.1
300	2.0	-	41.8

[1] The charge was heated to desired pressure, then immediately cooled to 0 psig.

At 75 psig and 1% SO_2, no significant changes in filtration rate or conversion of insoluble solids to soluble solids was noted after 10 minutes. At 125 psig and 1.5% SO_2, very little difference in conversion was noted between 20 minutes and 300 minutes.

All trial runs in the pilot plant investigation, unless otherwise noted, were therefore, treated for 15 minutes.

For our pilot plant investigations we have chosen a Hexagon Design. This design is both orthogonal and rotatable. Enough experimental points are represented as to be able to estimate all the coefficients in a second order model. This design is illustrated below.



For ease of calculation and identification, the variables are coded as follows:

$$x_1 = \frac{\text{SO}_2 \text{ concentration (\%)} - 2.25}{2.25}$$

$$x_2 = \frac{\text{Pressure (psig)} - 75}{25}$$

This gives rise to the following table denoting the coded values and the actual levels of the variables studied.

Table 17

CODED VALUES AND ACTUAL LEVELS OF VARIABLES STUDIED

Design Units		Factor Levels	
x_1 (% SO_2)	x_2 (psig)	x_1 (% SO_2)	x_2 (psig)
-1	0	0	75
1	0	4.5	75
0.5	-0.866	3.38	53.4
-0.5	-0.866	1.12	53.4
0.5	0.866	3.38	96.6
-0.5	0.866	1.12	96.6
0	0	2.25	75

Waste water sludges, used in the investigation were kept under aerobic conditions by bubbling air through the samples. It was not possible to conduct all the above experiments in a single day, and thereby maintain a steady state experimental environment. We therefore elected to run each experiment over a period of two days in two sub-sets or blocks, each block comprising the experiments run on a given day. To reduce the biasing effect of other unidentified factors, the trials were run in random order within the block. The center point represented by the design units (0, 0) was run three times to give an estimate of experimental error as well as the contribution from each block. Therefore, the sub-sets run are illustrated in Table 18.

Table 18

EXPERIMENTAL DESIGN

Design Units		Factor Levels	
x_1 (% SO_2)	x_2 (psig)	x_1 (% SO_2)	x_2 (psig)
Block 1			
-1	0	0	75
0.5	-0.866	3.38	53.4
0.5	0.866	3.38	96.6
0	0	2.25	75
0	0	2.25	75
0	0	2.25	75
Block 2			
1	0	4.5	75
-0.5	0.866	1.12	96.6
-0.5	-0.866	1.12	53.4
0	0	2.25	75
0	0	2.25	75
0	0	2.25	75

The coefficients of a first or second order regression equations were determined(11). A regression analysis was made to determine if the regression equation so determined adequately describe the response surface.

Treatment of Activated Sludge

Activated sludge from four sewage plants in the North New Jersey area was treated with SO_2 at low pressure. These sewage plants and their locations were:

Little Ferry, New Jersey
Livingston, New Jersey
Madison-Chatham, New Jersey
Parsippany-Troy Hills, New Jersey

Characteristics of the activated sludge before and after treatment, conversion, filtration rates and the experimental design as summarized in Table 19. Graphs of the conversion contour surfaces are shown in Figures 3 through 6.

Table 19

CHARACTERISTICS OF ACTIVATED SLUDGE BEFORE AND AFTER TREATMENT

Analysis Before Treatment

	Little Ferry		Livingston		Madison-Chatham		Parsippany-Troy	
	Block 1	Block 2	Block 1	Block 2	Block 1	Block 2	Block 1	Block 2
Total Solids (%)	0.74	0.77	0.64	0.68	0.60	0.50	0.82	0.85
Soluble Solids (%)	0.06	0.09	0.08	0.09	0.03	0.04	0.04	0.06
pH	7.35	7.35	7.32	7.25	7.3	7.1	7.38	7.50
Filtration Rate (seconds to collect 50 ml)	69	85	138	486	341	223	48	75

Observations After Treatment

Factor Levels		% Conversion				Filtration Rate (sec)			
x_1 (%SO_2)	x_2 (psig)	Little Ferry	Living-ston	Madison Chatham	Parsip-pany-Troy	Little Ferry	Living-ston	Madison Chatham	Parsip-pany-Troy
				BLOCK 1					
0	75	30.9	36.9	43.6	28.3	16	12	5	23
3.38	53.4	28.8	28.9	28.4	24.6	19	39	10	156
3.38	96.6	40.6	40.9	44.2	34.7	4	4	3	6
2.25	75	33.4	37.0	33.6	26.4	8	6	4	22
2.25	75	32.3	35.9	34.6	27.8	9	6	4	25
2.25	75	32.6	37.9	34.6	27.5	8	6	4	20
				BLOCK 2					
4.5	75	37.0	32.8	38.6	33.4	6	5	4	13
1.12	96.6	30.8	32.1	39.7	33.4	6	5	3	11
1.12	53.4	22.5	27.4	36.2	18.6	4	173	82	2533
2.25	75	29.4	31.8	33.2	29.4	9	7	4	41
2.25	75	30.8	31.6	34.0	25.7	8	8	4	45
2.25	75	30.2	31.9	33.8	30.2	8	7	4	45

Discussion

Filtration Rate: Efforts to fit first or second order regression equations to describe filtration rates were unsucessful. An unknown factor is effecting this parameter. The anomality observed and investigation as to exact nature of the anomality is beyond the scope of this investigation. However, we can advance several theories for this behavior: (1) under the varied reaction condition, molecular weights of the solubilized solids differ, (2) the "soluble solids" may be colloidal and have differing particle size under different treatment conditions, (3) the method for measuring filtration rates is inadequate as a measure of "filterability".

In general, the best filterability is obtained under conditions giving maximum conversion where alternate conditions for maximum conversion are possible, best filterability occurs under condition of higher SO_2 concentration.

Conversion: With all four plants studied, the effect of both pressure and SO_2 concentration is significant. Conversion contour surfaces, however, differed for each plant.

Treatment of activated sludge from the Little Ferry, New Jersey plant gave a maximum of about 41% conversion within the ranges of the factors studied. Based on the data obtained from the Hexagon Experimental Design as shown in Table 19, SO_2 concentration, pressure effects and the interaction term are significant.

The second order regression equation (in design units)

$$Y = 31.45 + 4.72x_1 + 5.80x_2 + 2.50x_1^2 - 1.87x_2^2 + 2.02x_1x_2$$

adequately describes the response surface. Figure 3 represents contour lines of 25%, 30%, 35% and 40% conversion yields. That is, any point on the yield contour surface represents a combination of pressure and SO_2 concentration which will give that identical yield. The inscribed hexagon represents the actual yields obtained in our experimental work. A analysis of variance table for the coefficients obtained is shown in Table 20.

Figure 3

YIELD CONTOUR SURFACES

(Activated Sludge from Little Ferry, N. J.)

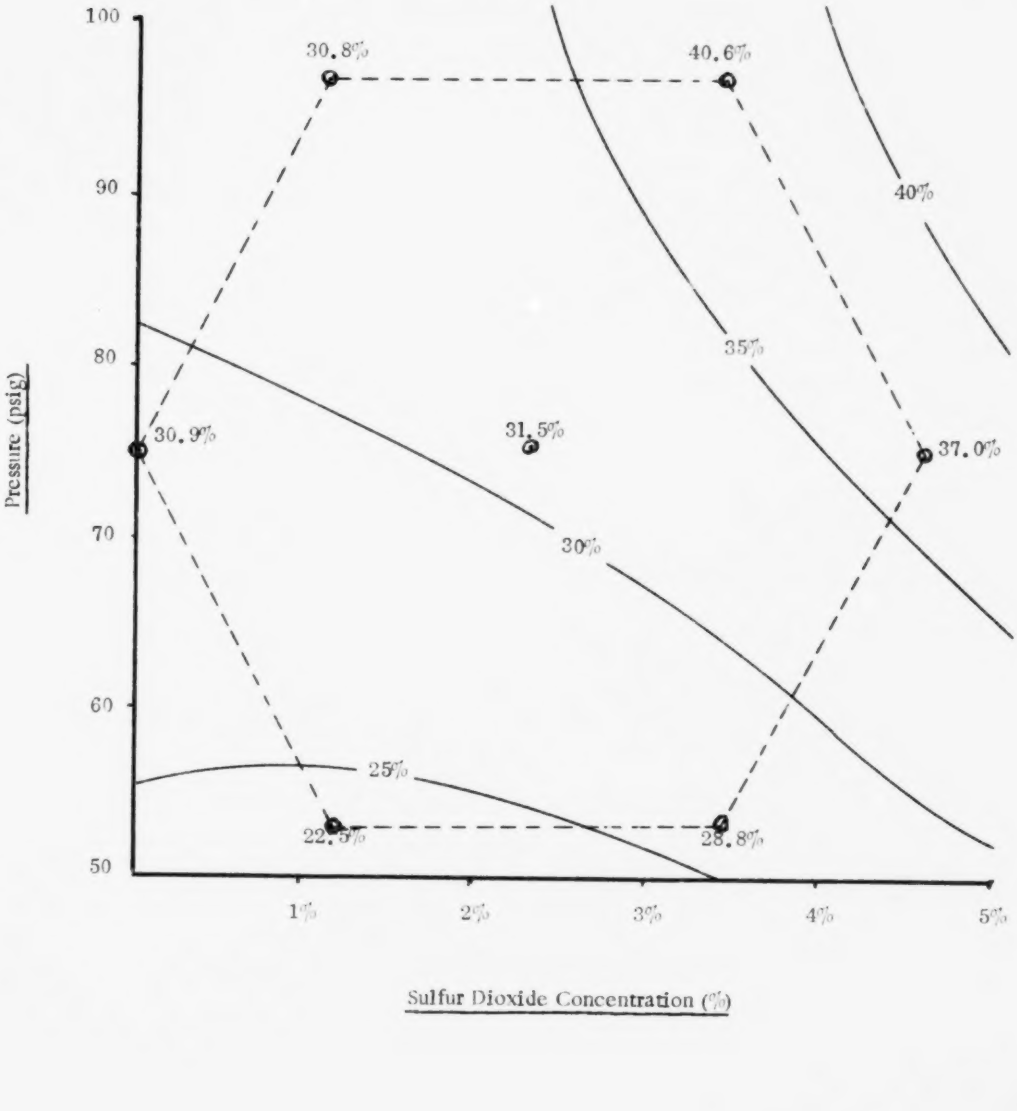


Table 20

REGRESSION ANALYSIS TABLE FOR CONVERSION
(Little Ferry, New Jersey)

Source of Variance	Sum of Sq.	D.F.	M.S.	F Ratio
Blocks	26.7006	1	26.7006	130.77*
B_1	66.7408	1	66.7408	326.88*
B_2	101.002	1	101.002	494.69*
Quadratic	14.6009	2	7.3005	35.76*
B_{12}	3.0625	1	3.0625	15.00*
Lack of fit	1.1850	1	1.1850	5.80 N.S.
Error	0.8167	4	0.2042	

* 5% value of $F_{1,4} = 7.71$; $F_{2,4} = 6.94$

Between 50 psig and 60 psig, increasing SO_2 concentration, up to about 2% appears, to be detrimental to conversion. As SO_2 concentration is increased beyond this level, there is a slow increase in conversion. For example, at 55 psig and no SO_2 conversion yields are 25%. On adding SO_2, yields decrease slightly up to about 1.3% SO_2, then increase slowly to about 31% at an SO_2 level of 5%.

Above 60 psig, addition of any amount of SO_2 increases yields.

Another interpretation of Figure 3 is that by use of SO_2, pressure can be decreased to obtain the same or higher yields. For examples, if one observes the 35% contour surface, it can be seen that if one increases the SO_2 concentration from 2.3% to 5%, then the treatment pressure can be lowered from 100 psig to 64 psig.

Treatment of activated sludge from Livingston, New Jersey gave a maximum conversion of about 41%. Based on the data shown in Table 19 and the regression analysis shown in Table 21, pressure, the quadratic term and interaction term are significant. A second order equation (in design units)

$$Y = 34.35 + 0.35x_1 + 4.82x_2 + 0.50x_1^2 - 2.87x_2^2 + 4.21x_1x_2$$

adequately describes the response surface. Figure 4 represents contour lines for 25%, 30% and 40% conversion yields.

Figure 4

YIELD CONTOUR SURFACES

(Activated Sludge from Livingston, N.J.)

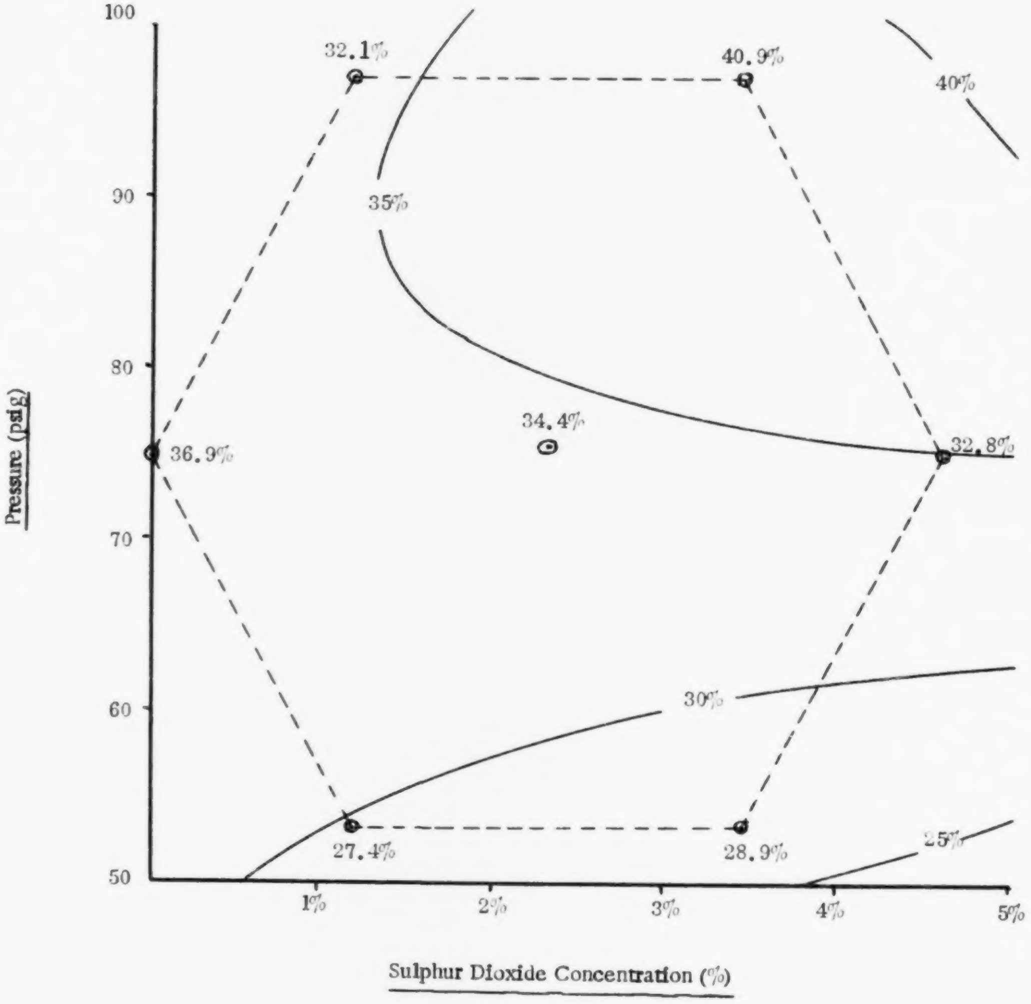


Table 21

REGRESSION ANALYSIS TABLE FOR CONVERSION

(Livingston, New Jersey)

Source of Variance	Sum of Sq.	D.F.	M.S.	F Ratio
Block	74.5016	1	74.5016	239.6898*
B_1	0.3675	1	0.3675	1.1823
B_2	19.7225	1	6..7225	224.314*
Quadratic	12.7008	2	6.3504	20.4308*
B_{12}	13.3225	1	13.3225	42.8617*
Lack of fit	0.9107	1	0.9107	2.9299 N.S.
Error	1.2433	4	0.3108	

* 5% value for $F_{1 \cdot 4} = 7.71$; $F_{2 \cdot 4} = 6.94$

Pressure is a significant factor. The significance of the SO_2 x pressure interaction term indicates conversion differs at different levels of SO_2 concentration and pressure. For example, at pressure below 80 psig, addition of SO_2 decreases conversion; at 80 psig, addition of SO_2 makes little difference; above 80 psig, addition of SO_2 improves soluble solids formation.

It should also be noted that at SO_2 concentrations below about 1%, increasing pressure decreases conversion yields.

Treatment of activated sludge from Madison-Chatham, New Jersey gave a maximum conversion of about 44%. Based on the data obtained as shown in Table 19, both SO_2 concentration and pressure are highly significant as is the interaction term. The second order regression equation (in design units)

$$Y = 33.97 - 2.22x_1 + 5.57x_2 + 7.13\,x_1^2 + 1.83x_2^2 + 7.10x_1x_2$$

adequately describes the response surface. Figure 5 presents the 30%, 35%, 40% and 45% contour curves for conversion yield. An analysis of variance table for the coefficients obtained is shown in Table 22.

Figure 5

(Activated Sludge from Madison-Chatham, N.J.)

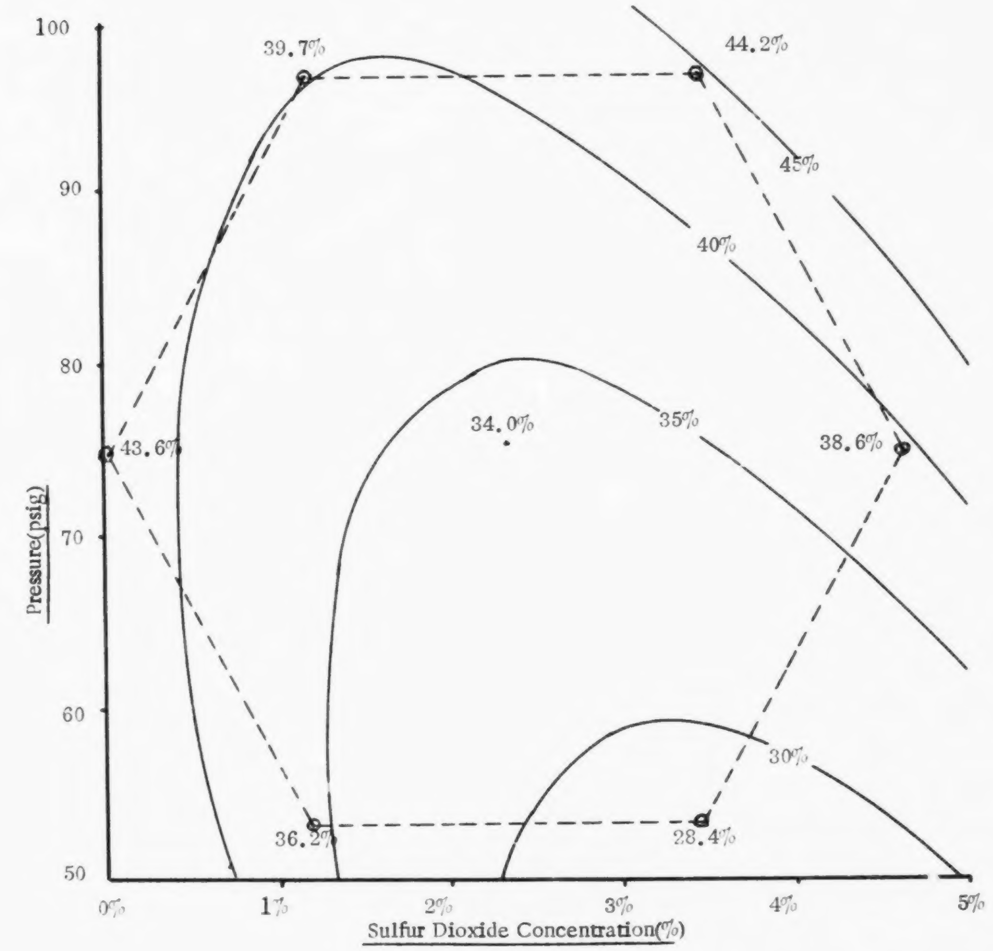


Table 22

REGRESSION ANALYSIS TABLE FOR CONVERSION
(Madison-Chatham, New Jersey)

Source of Variance	Sum of Sq.	D.F.	M.S.	F Ratio
Blocks	1.0212	1	1.0212	8.0632
Linear				
b_1	14.7408	1	14.7408	116.39*
b_2	93.1225	1	93.1225	735.274*
Quadratic	81.3672	2	70.6836	321.229*
Interaction				
b_{12}	37.8225	1	37.8225	298.638*
Lack of Fit	0.5078	1	0.5078	4.0098 N.S.
Error	0.5066	4	0.12665	

* 1% value for $F_{1,4} = 21.2$; $F_{2,4} = 18.0$

Figure 5 is useful in selecting alternate conditions to obtain specific conversions. For example, at pressures below 100 psig addition of SO_2 causes conversion to go through a minimum which is most pronounced at lower pressures. Also, one has a choice of either high or low SO_2 concentration (keeping pressure constant) to give identical conversions.

Treatment of activated sludge from Parsippany-Troy Hills, New Jersey gave a maximum conversion of about 35%. Based on the data obtained and shown in Table 19 and regression analysis shown in Table 23, both pressure and SO_2 concentration are significant. A first order equation (in design units)

$$Y = 28.33 + 3.60x_1 + 7.21x_2$$

adequately describes the response surface. Figure 6 presents contour curves for 20%, 25%, 30% and 35%.

Figure 6

YIELD CONTOUR SURFACES

(Activated Sludge from Parsippany-Troy Hills, N. J.)

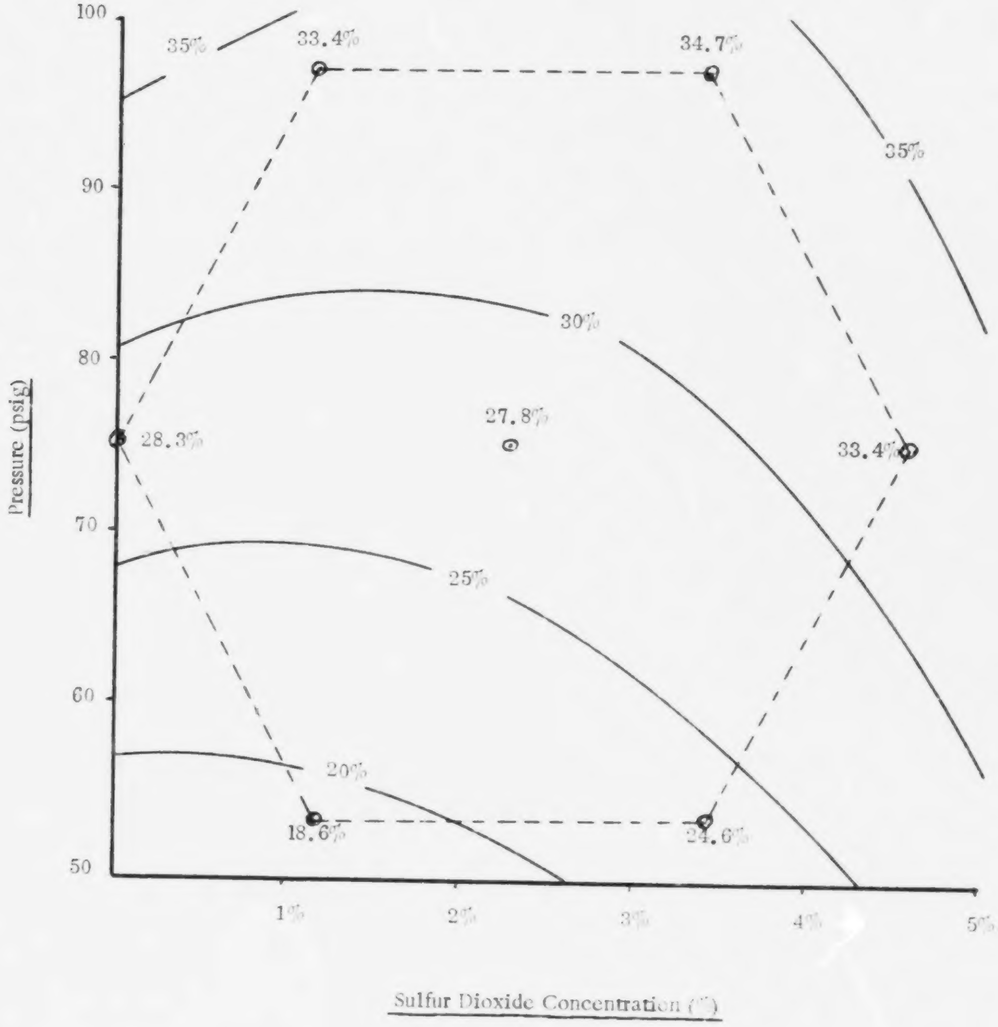


Table 23

REGRESSION ANALYSIS TABLE FOR CONVERSION

(Parisppany-Troy , New Jersey)

Source of Variance	Sum of Sq.	D.F.	M.S.	F Ratio
Blocks	0.1638	1	0.1633	
b_1	39.88	1	38.88	24.6607*
b_2	155.8276	1	155.8276	98.8378*
Lack of Fit	15.66	4	3.9150	2.4832
Error	6.3066	4	1.5766	

* 5% value for $F_{4,4} = 6.39$

At low pressure, addition of SO_2 has a dramatic, beneficial effect on increasing conversion. However, as pressure increases, there is an area where addition of SO_2 actually decreased conversion. Then as SO_2 concentration gets above a certain level, increased conversion is again favored.

Mixed Sludge

Heat treatment of mixed sludge, alone or in the presence of SO_2, has not previously been explored. This section summarizes the results of investigations relating to the conversion of the solids present and improvement in the filterability of mixed sludge.

Procedure

All treatments were conducted in the 5-gallon reactor described in the previous pages.

Conversion yields and filtration rates were measured in a manner similar to that previously described.

Selection of Variables

Three variables were considered important in this process--time of treatment, pressure and SO_2 concentration.

Pressure and SO_2 levels were in the ranges of 50 to 100 psig and 0 to 5% respectively. We suspected that treatment time would be longer than that previously used. Mixed sludge (approximately 90% primary sludge and 10% activated sludge) obtained from Madison-Chatham, N.J. was treated with SO_2 at 75 psig for 15, 30 and 240 minutes. As shown in Table 24, little change in either conversion or filterability was observed after 30 minutes.

Table 24

PRIMARY SLUDGE TREATED WITH SO_2 AT LOW PRESSURE
(Madison-Chatham, New Jersey)

Analysis of Raw Primary Sludge Before Treatment:

	Sample 1	Sample 2
Filtration Rate (seconds)	401	375
pH	6.2	6.7
Total Solids (%)	1.4	0.9
Soluble Solids (%)	0.8	0.7

Treatment			Measurements	
SO_2 Conc. (%)	Time (min)	Pressure (psig)	Filtration Rate (sec.)	Conversion (%)
		Sample 1		
0.0	15	75	11	8.17
2.5	30	75	3	29.3
		Sample 2		
2.5	15	75	4	23.5
2.5	240	75	2.5	29.2

Subsequently, mixed sludge from three local sewage plants-- Little Ferry, New Jersey, Livingston, New Jersey and Parsippany-Troy Hills, New Jersey--- were treated for 30 minutes in the 5-gallon reactor at several levels of pressure and SO_2 concentration using the Hexagon Experimental Design described in our work with activated sludge. Characteristics of the mixed sludge, before and after treatment are shown in Table 25 and graphs of various conversion contour surfaces for each sample are shown in Figures 7 through 9.

Table 25

CHARACTERISTICS OF MIXED SLUDGE BEFORE AND AFTER TREATMENT

Analysis Of Mixed Sludge Before Treatment

	Little Ferry		Parsippany		Livingston	
	Block I	Block II	Block I	Block II	Block I	Block II
Total Solids (%)	4.64	4.63	3.83	3.79	3.14	3.00
Soluble Solids (%)	0.15	0.21	0.10	0.13	0.13	0.13
pH	6.40	6.10	6.40	6.05	6.40	6.10
Filtration Resistivity (seconds to collect 50 ml)	3529	3960	942	1129	1518	1542

Observations After Treatment

	Factor Levels		% Conversion			Filtration Resistivity (sec)		
	X_1 (%SO_2)	X_2 (psig)	Little Ferry	Parsippany	Livingston	Little Ferry	Parsippany	Livingston
				BLOCK I				
1	0	75	13.5	14.5	22.6	2068	60	20
2	3.38	53.4	16.2	15.1	19.0	2900	1484	2484
3	3.38	96.6	23.4	24.0	27.7	149	28	42
4	2.25	75	18.6	17.9	23.5	610	321	137
5	2.25	75	18.2	17.9	22.8	1130	407	373
6	2.25	75	19.3	18.9	23.2	148	182	336
				BLOCK II				
1	4.5	75	19.6	22.2	24.2	3311	90	187
2	1.12	96.6	18.2	19.8	24.6	1414	20	20
3	1.12	53.4	12.6	12.7	14.9	2564	1245	3455
4	2.25	75	19.8	17.6	22.6	966	156	131
5	2.25	75	19.6	18.1	24.2	631	322	121
6	2.25	75	17.9	18.6	24.6	331	276	93

Discussion

Filtration Rate: No representative regression equations could be obtained. In general, filterability (as measured) was best in areas showing highest conversion.

Conversion: Conversions for the three plants ranged from 24% to 28%.

Treatment of mixed sludge from Little Ferry, New Jersey, within the ranges of the variables studied, gave a conversion of about 24%. Based on the data presented in Table 25 both SO_2 concentration and pressure are significant factors. A second order equation (in design units)

$$Y = 18.9 + 3.5x_1 + 3.70x_2 - 2.35x_1^2 - 0.95x_2^2 + 0.92x_1x_2$$

adequately describes the response surface. Figure 7 presents contour lines for 10%, 15% and 20% conversion yields. A regression analysis for the coefficients of the equation is summarized in Table 26.

Table 26

REGRESSION ANALYSIS TABLE FOR CONVERSION
(Little Ferry, New Jersey)

Source of Variance	Sum of Sq.	D.F.	M.S.	F Ratio
Blocks	0.1875	1	0.1875	0.5300
b_1	36.7500	1	36.7500	103.887*
b_2	40.9600	1	40.9600	115.788*
Quadratic	9.6373	2	4.8186	13.6216*
b_{12}	0.6400	1	0.6400	1.8092
Lack of Fit	2.6526	1	2.6526	7.4985
Error	1.4150	4	0.3538	

*5% value for $F_{1 \cdot 4} = 7.71$; $F_{2 \cdot 4} = 6.94$

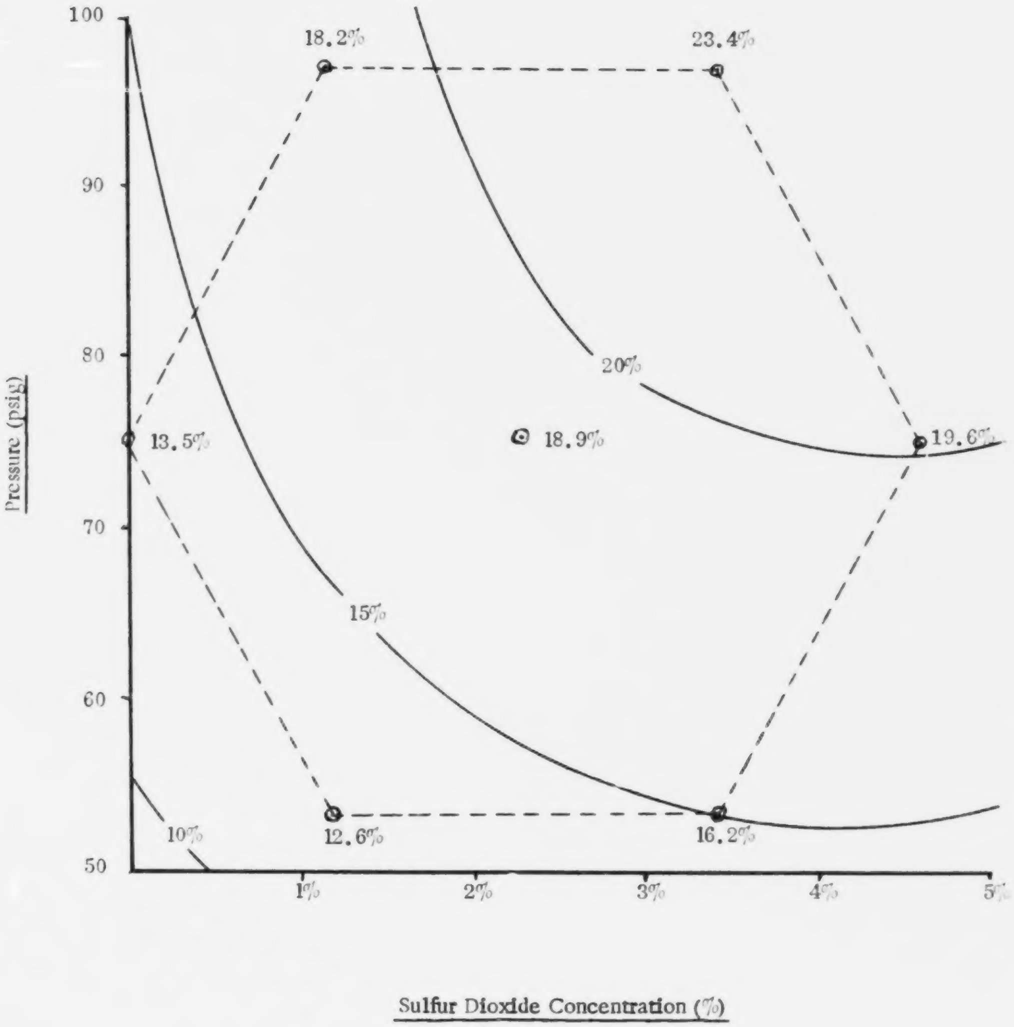
With no SO_2, conversion can be increased from approximately 10% to 15% by increasing pressure from 50 psig to 100 psig. However, conversion of mixed sludge containing 4% SO_2 can be increased from 14% to 24% by increasing pressure from 50 psig to 100 psig.

Mixed sludge from Parsippany-Troy Hills, New Jersey can be converted

Figure 7

YIELD CONTOUR SURFACES

(Mixed Sludge from Little Ferry, N.J.)



to the extent of about 21%. Both SO_2 and pressure are significant factors. A first order regression equation

$$Y = 18.11 + 3.67x_1 + 4.62x_2$$

adequately describes the response surface. Figure 8 presents contour curves for 15%, 20% and 25% conversion. A regression analysis table for the coefficients estimated is shown in Table 27.

Table 27

REGRESSION ANALYSIS TABLE FOR CONVERSION
(Parsippany-Troy, New Jersey)

Source of Variance	Sum of Sq.	D.F.	M.S.	F Ratio
Blocks	.0408	1	.0408	0.3041
b_1	40.3337	1	40.3337	300.6612*
b_2	63.9967	1	63.9967	477.0533*
Lack of Fit	1.9486	4	.4872	3.6318
Error	0.5366	4	.13415	

* 5% value of $F_{4 \cdot 4} = 6.39$

Both SO_2 and pressure effects are significant, with pressure having slightly more influence on conversion. With no SO_2 present, increasing pressure from 50 psig to 100 psig increases conversion from 16% to 27% at 50 psig and 100 psig respectively.

Attempts to fit a regression equation to the data for mixed sludge from Livingston, New Jersey were unsuccessful as shown in Table 28. However, the general shape of conversion contours of 15%, 20% and 25% is shown in Figure 9. It appears that pressure is the most important factor, although the presence of SO_2 does improve conversion at any level of pressure. The equation representing these contour curves is

$$Y = 23.48 + 1.73x_1 + 5.31x_2 - 0.08x_1^2 - 2.55x_2^2 - 0.58x_1x_2$$

Figure 8

YIELD CONTOUR SURFACES

(Mixed Sludge from Parsippany-Troy Hills, N. J.)

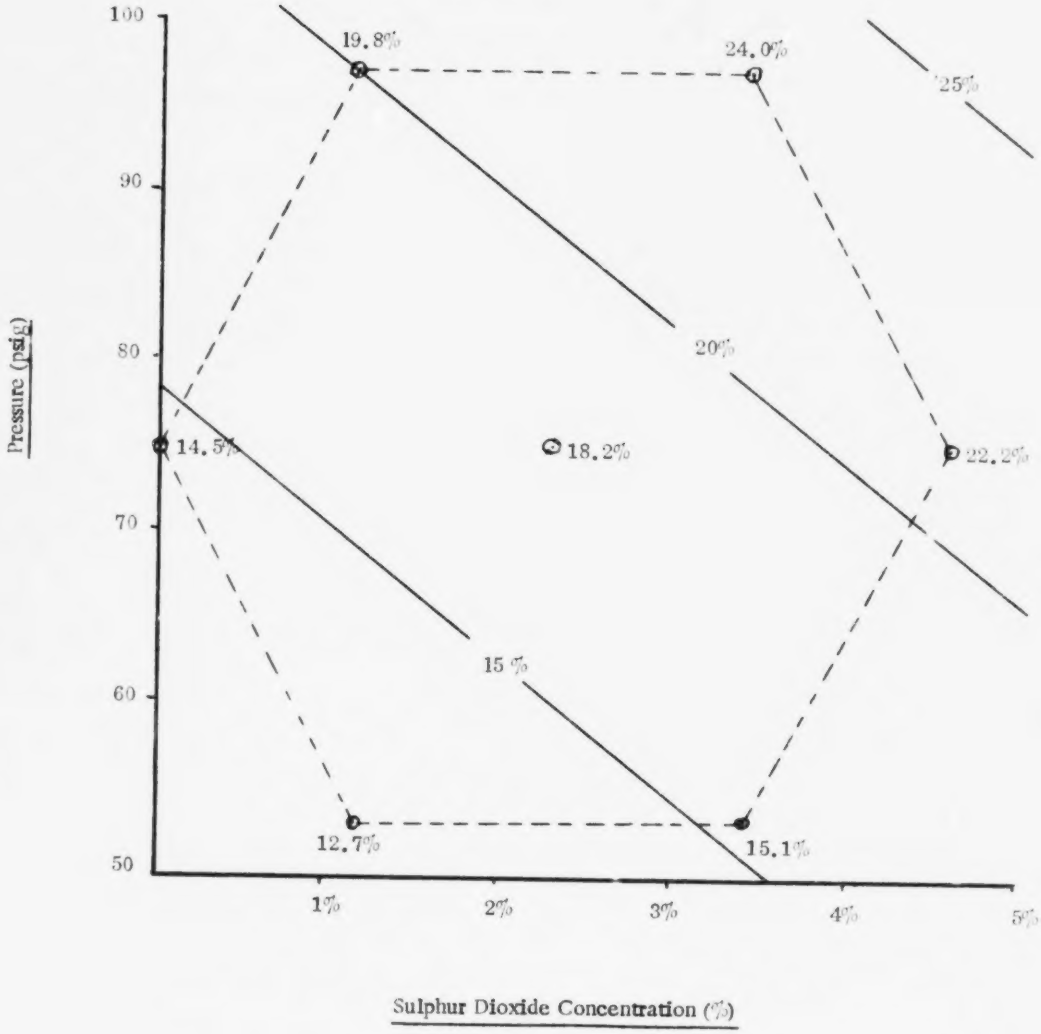


Figure 9

YIELD CONTOUR SURFACES

(Mixed Sludge from Livingston, New Jersey)

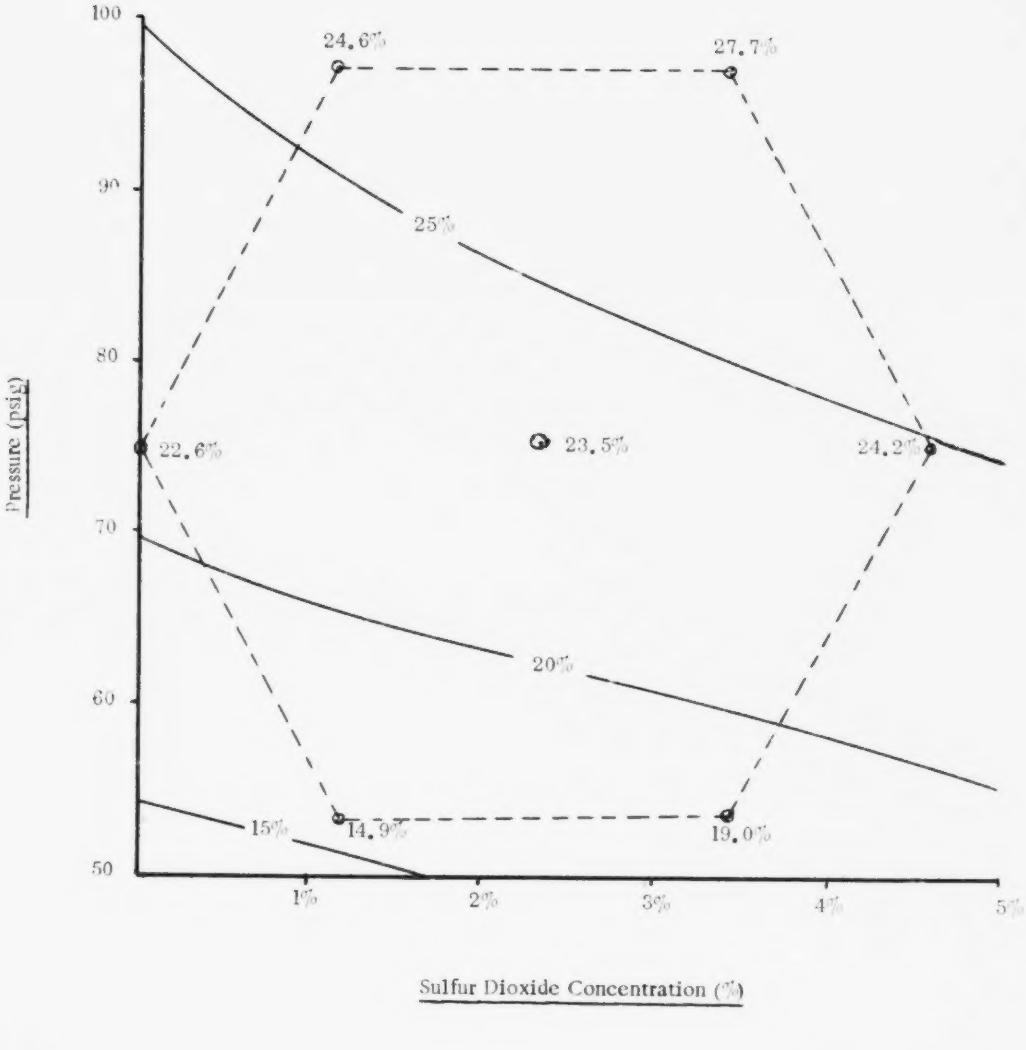


Table 28

REGRESSION ANALYSIS TABLE FOR CONVERSION
(Livingston, New Jersey)

Source of Variance	Sum of Sq.	D.F.	M.S.	F Ratio
Blocks	1.1407	1	1.1407	3.6659
b_1	9.0133	1	9.0133	28.9981
b_2	84.6400	1	84.6400	272.3080
Quadratic	9.7637	2	4.8818	15.7061
b_{12}	0.2500	1	0.2500	0.8043
Lack of Fit	5.9314	1	5.9314	19.0829*
Error	1.2433	4	0.3108	

* Significant at the 5% level

PILOT PLANT STUDIES - LARGE SCALE

This section of the report deals with aspects of engineering and economics that were developed by operating this process with larger scale equipment including a 200-gallon reactor.

The results of this work may be simply summarized as follows

> The conditioning of activated sludge with SO_2 at low pressure has been demonstrated under pilot plant conditions having processed about 300,000 lbs of raw feed material.
>
> Feasible process conditions have been established for all the required unit operation and unit process steps for production of both an OFC containing 50% solids and spray dried powder.
>
> A conservative projection of investment and operating costs is presented to facilitate technical/economic evaluation of the product OFC.
>
> Further engineering work is needed to optimize process economics and advance engineering approaches to product quality control.

For clarity, the remainder of this section is presented under the following headings

- Basis for Pilot Plant Work
- Pilot Plant Experience
- Cost Estimates
- Need for Further Work

Basis for Pilot Plant Work

Pilot plant work was conducted in-house, using our own laboratory data with small equipment and with the requirement to produce approximately 200 lbs of OFC within an allowable time span for animal feeding tests. Work was initiated on an expedited basis because it was not possible to use an outside contractor for this service as had been planned.

The selected contractor suffered a major equipment failure and difficulty was experienced in finding an acceptable, qualified substitute.

The bulk of the necessary equipment and all the needed utilities were readily available in our own high-boy pilot plant facility. We were able to locate additional used process equipment which would be adequate, though not ideal, for the service. We expedited completion of an adequate process equipment train at our facility to minimize further time loss.

The OFC production requirement dictated processing as much as 300,000 lbs of activated sludge because of its low solids concentration.

The operational limitations of the pilot plant, together with OFC production requirements, lead to an emphasis on manufacturing. Nevertheless, feasible process conditions were demonstrated. These may not necessarily represent optimum conditions.

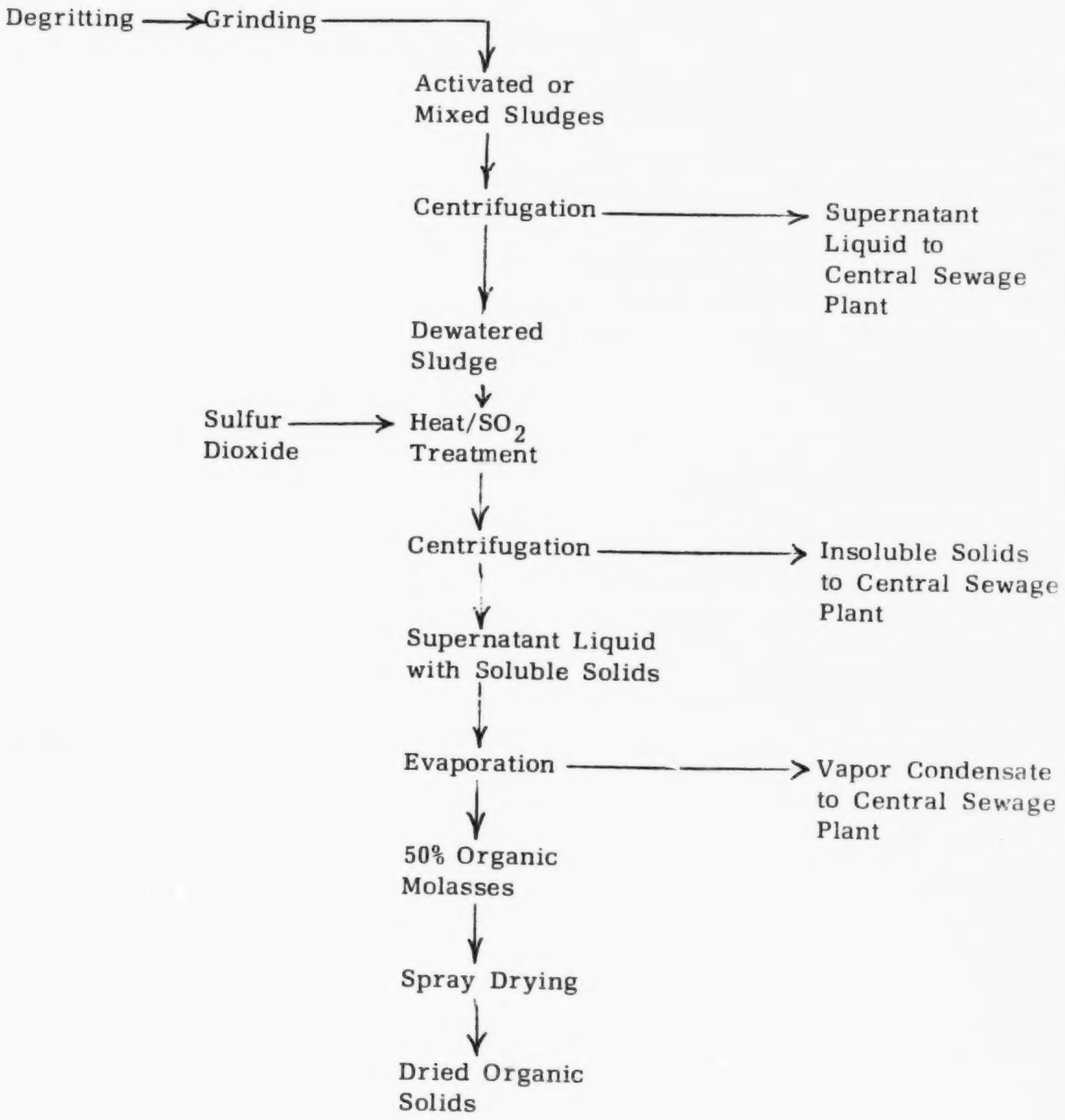
Pilot Plant Experience

Equipment and Conditions

Pilot plant demonstration was accomplished according to the flow scheme shown in Figure 10. Figure 11 presents a

FIGURE 10

FLOW DIAGRAM FOR HEAT/SO_2 TREATMENT OF SLUDGE


Degritting
Grinding
Activated or Mixed Sludges
Centrifugation
Supernatant Liquid to Central Sewage Plant
Dewatered Sludge
Sulfur Dioxide
Heat/SO2 Treatment
Centrifugation
Insoluble Solids to Central Sewage Plant
Supernatant Liquid with Soluble Solids
Evaporation
Vapor Condensate to Central Sewage Plant
50% Organic Molasses
Spray Drying
Dried Organic Solids

quantitative flow diagrammatic which includes representative temperatures, feasible pressures and flow rates used in later cost calculations.

Equipment used was minimal consisting of a 2,000-gallon settling tank, a 1,800-gallon holding tank, a 250 gallon, 250 psi WP, agitated, jacketed reactor, a Model BRPX-207-SVG-39 DeLaval Special Sludge Concentrator and various pumps.

Reactor agitation was provided by a 3" impellor, driven at 180 rpm by a 2 HP motor.

. Operational Experience

- Sludge Feed Material

Activated sludge was obtained from Parsippany-Troy, New Jersey Sewage Treatment Plant because it contained no industrial wastes and was only a few miles from our facility. It was received in 2,000-gallon tank truck loads. The solids concentration was between 0.2 and 0.3% (necessitating processing a total of about 40,000 gallons of sludge). It was used immediately, as fresh sludge, to avoid an anaerobic condition in the feed stream.

- Thickening

Raw activated sludge was pumped to a holding tank and allowed to settle for 30 minutes. The supernatant liquid was pumped to a sewer.

The settled sludge was centrifuged at a rate of 450 GPH and the solids discharged into a holding tank at 12-15% solids and held there under aerobic conditions until sufficient sludge was available to pump a full batch quantity to the reactor. Approximately 8 hours was required to obtain 200 gallons of this material.

Difficulty was encountered in pumping the 12-15% material and it became necessary to dilute it to 6-8% with water before pumping to the reactor.

. SO_2 Treatment

200 gallons of 6-8% activated sludge was pumped to the reactor, SO_2 was added at the 5% level (based on solids content). Agitation was started, steam fed to the jacket and internal pressure brought up to 100 psig, held at this pressure for 30 minutes, then cooled to 100°F and discharged into the holding tank. This procedure was repeated 12 times. Conversion of insoluble solids to soluble solids under these conditions was 35-40%.

. Separation of Insolubles

The treated sludge was passed through the centrifuge at 100°F (the upper temperature of the centrifuge is 125°F). The centrifuge clogged repeatedly.

. Concentration of OFC

The liquors separated above contained about 8% soluble solids. This was concentrated to 20% solids by batch stripping using the reactor as the pot.

The condensate had a putrid odor and should be deodorized if it is to be discharged to surface water. Analysis of this condensate was as follows

Table 29

ANALYSIS OF DISTILLATE

Total volitile solids @60°C	99.92%
BOD	20 mg/l
COD	1,000 mg/l
Ammonia (as N)	30 mg/l
Kjeldahl N	32 mg/l

. Spray Drying OFC

Laboratory experiments in our pilot spray drying equipment indicated that the OFC could be spray dried to a flowable powder. The capacity of this spray dryer was too small to process requisite quantities of OFC rapidly enough.

Subsequently, the OFC was taken to Nichols Engineering & Research Corporation, Belle Mead, New Jersey, for spray drying. The equipment used was a Nichols/Niro Pilot Spray Drying, 7-1/2' diameter, 7-1/2' cylindrical height: 60° cone bottom; type F-10 centrifuged atomizer equipped with a HiVane atomizing wheel; two point product collection at chamber bottom and cyclone separators, respectively; stainless steel feed tank and feed line; Moyno feed pump; Ohaus Moisture Balance; B&L binocular microscope, 150x.

Test results are as shown in Table 30.

. Discussion of Results

- Settling

Visual inspection of the homogeneity of settling was difficult but it appeared, on occasion, that layers of water became interspersed within the settled sludge. It is probable that low rate rake agitation would decrease settling time requirements and increase solids concentration and homogeneity of the underflow.

Table 30

SPRAY DRYER TEST RESULTS

RUN DATA DRUM #	1				2	3	
CONDITIONS	A	B	C	D		A	B
Inlet air temp (^{O}F)	310	325	325	325	312	312	312
Outlet air temp (^{O}F)	175	195	153	187	189	198	194
Length of run (mins)	12	45	15	30	124	71	55
Atomizing wheel speed (rpm)	23800	------	------	22300	------	------	------
Feed rate (est.gph)*	19.7	20.5	27.1	21.8	19.3	18.4	19.9
Residence time (secs)	16.9	16.7	16.1	14.1	14.0	14.0	13.5
PRODUCT DATA							
Residual Moisture (%)	-	2.1	-	2.6	2.3	2.1	2.2
PRODUCT SPLIT (lbs)							
Chamber	------	18-3/4	------	------	19	17-3/4	
Cyclone	------	19-1/4	------	------	37-3/4	38	
Sweepings		-			-	15	

* estimate based on 40 gallons/drum of feed.

- Slurry Pumping

Positive displacement, sludge pumps such as Moyno and Falk were unavailable to us. This equipment is capable of handling heavy sludges approaching the 12-15% solids concentration obtained after centrifugation. Optimum solids concentration in the reactor feed is probably below this level so that future pump selection should not be a problem.

- Reactor Heating

Steam was fed from a 30 HP boiler to the jacket and internal coils of the reactor. Minor improvements were possible by insulating the steam header, repiping in the vicinity of the reactor to introduce steam to the jacket at multiple points and lagging of the jacket.

Despite these steam system deficiencies, full output of the boiler permitted heating a 200-gallon reactor charge of city water to 100 psi in 30 minutes. In contrast, 4 hours were required with a 200-gallon charge of sludge reaction mass. The increase in density (heat capacity) cannot account for this so that instead one looks to heat transfer and agitation considerations. These are well known to be troublesome with sludge.

The imperative for production of OFC did not permit pilot plant modification. Further, the operational necessity for two shift operations did not permit isolation of heat transfer through the jacket separately from the coil with the agitation that was provided because both surfaces were simultaneously required to hold the period to a reasonable time.

- Product Quality and Quality Control

Complete analysis were not conducted on a day to day basis but rather the product was blended and analyzed as a blend. This tended to mask the day to day variations which may have taken place.

Analysis of the product for heavy metals was conducted after completion of pilot plant operations and their presence was indicated even though the sludge source was domestic sewage. No attempt was made to remove these metals by treatment in the pilot plant. The fact that the entire processing system was constructed of stainless steel or epoxy-coated carbon steel suggests there was no metal pickup from the system.

Cost Estimates

Based on our experience in the pilot plant we present:

- preliminary process design for a demonstration plant capable of producing 900 tons of 50% OFC per year.
- the capital investment required for the above plant.
- manufacturing costs.

The purchased equipment cost was obtained from manufacturers, while installations, utilities and indirect costs were taken as a percentage of purchased equipment cost.

The feasibility of an alternate plant producing 450 tons of dry solids per year was also investigated. The design is essentially the same as that of the basic plant, but evaporation is conducted to 20-25% solids. The costs calculated have been maximum (conservative). We have not permitted any consideration of the value of removal of the sludges, BOD and COD, reduction of solids handling which would be a negative cost factor, nor savings which will accrue with identification and use of higher performing equipment such as pumps, centrifuges and heat exchange equipment.

The design and economic analysis were based on the following assumptions:

1. The demonstration plant is an extension of an existing sewage treatment plant. Thus no cost was assigned for buildings, yard improvements, service facilities and land.

2. The plant receives, at no cost from the central sewage plant, thickened activated sludge containing 3% total solids.

3. All discharges (liquids and solids) are handled at no cost by the central sewage plant.

4. No cost was assigned for the administration of this plant. It is assumed that the administrative expenses of the central sewage plant will absorb the expenses of this adventure.

5. The capital investment statement incorporates no time value of money.

6. The purchased equipment will be depreciated over a 12 year period with no salvage value.

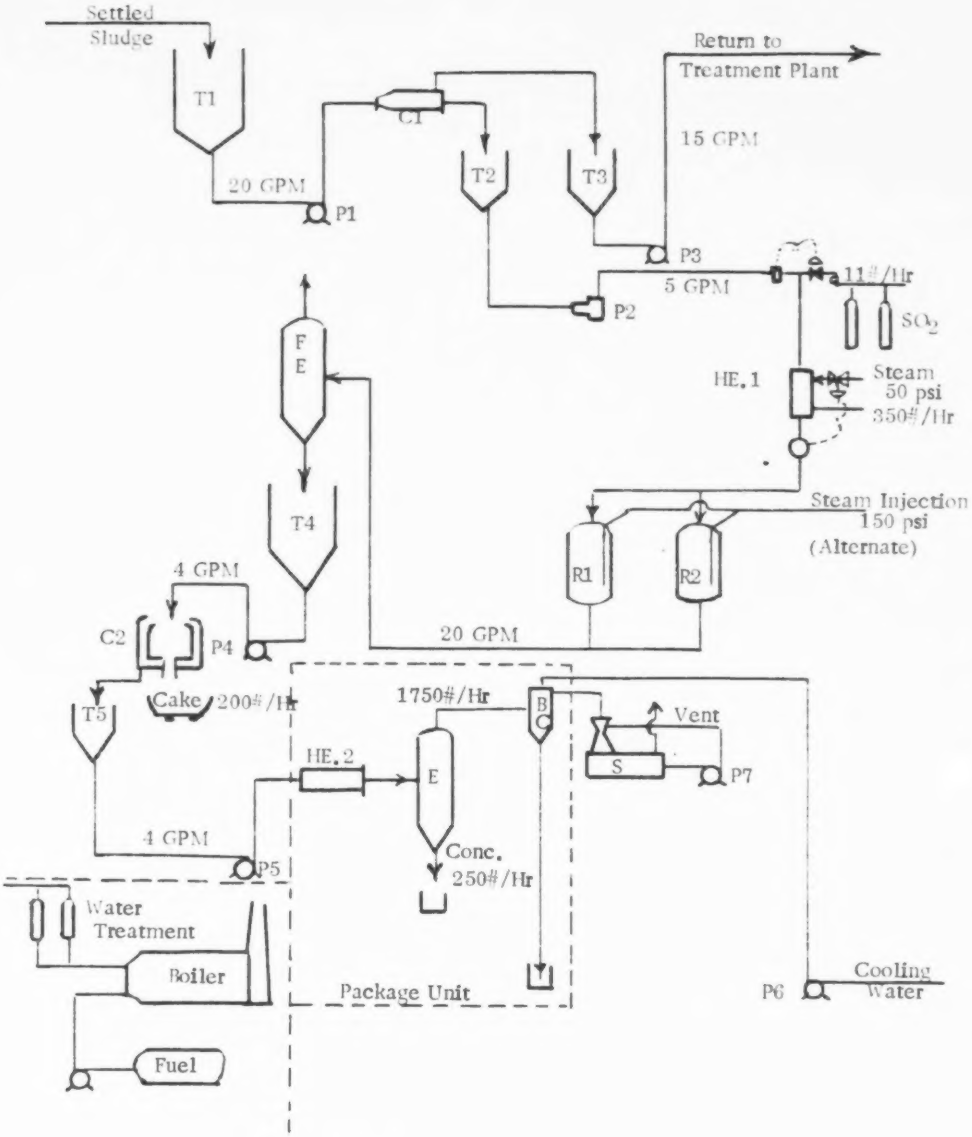
7. The plant will be tax-exempt.

8. The existing laboratory of the central sewage plant, will handle, at no cost, the needs of this operation.

9. Plant will operate 20 hours per day, 300 days per year.

Figure 11

PROCESS FLOW SHEET


Settled Sludge
T1
20 GPM
P1
C1
T2
T3
Return to Treatment Plant
15 GPM
P3
5 GPM
P2
11#/Hr
SO_2
HE. 1
Steam 50 psi 350#/Hr
Steam Injection 150 psi (Alternate)
R1
R2
F E
T4
4 GPM
C2
P4
20 GPM
Cake 200#/Hr
T5
1750#/Hr
B
Vent
S
P7
HE. 2
E
4 GPM
P5
Conc. 250#/Hr
Water Treatment
Boiler
Package Unit
Fuel
P6
Cooling Water

OFC Manufacturing Plant

The process adopted is outlined schematically in Figure 11. Figure 11 is a quantitative flow diagram describing the quantities and the conditions employed in the process.

The reactor consists of a continuous pressure plate heat exchanger equipped with a holding tank. The holding time is 15 minutes at 350°F. The treated sludge is separated using a continuous decanter centrifuge, and the cake having 50% moisture is disposed by the central sewage plant.

The list of equipment and the estimated costs are shown in Tables 31 and 32.

The purchased equipment cost for a plant producing 900 tons of 50% OFC is estimated at $127,000. The cost of a plant with a spray drying unit is estimated at $154,000.

Estimated capital investment for both liquid OFC and the spray dried OFC plants are presented in Tables 33 and 34. These are $223,000 for the OFC plant and $273,000 for the dry solids plant.

The estimated manufacturing cost of a pound of 50% OFC is 6.1¢ or less (Table 35) and a pound of spray dried solids is 13.2¢ or less (Table 36).

Table 31

ESTIMATED EQUIPMENT COST: 900 TPY - 50% OFC PLANT

T1	Holding Tank - 750 Gal. C.S.	1,000
P1	Centrifuge Feed Pump 17 GPM 25'	500
C1	Dewatering Centrifuge Pfaudler Z2L	23,000
T_2T_3	Holding Tanks 300 Gals. C.S.	1,200
P_2	Moyno Positive Displacement Pump 5 GPM	1,000
P_3	Centrifugal Pump 12 GPM - 75'	500
HE-1	Heat Exchanger 20 sq. ft. Plate on Equip. with controls	2,000
A	SO_2 Addition System - with controls	3,000
R,R_2	Reactors 200 psi 400 Gals. 316 SS	5,000
FE	Flash Evaporator Shell	2,000
T4	Holding Tank 500 Gals 316 SS	1,000
C_2	Continuous Solid Discharge Centrifuge	16,000
T5	Holding Tank 500 Gals. 316 SS	1,000
E	Evaporator with controller, instruments feed pump, (P5) preheater (HE-2). Barometric condenser	29,0[illegible]
Steam Boiler	Packaged Unit 10,000 #/Hr. 150 psi with Instrumentation. Fuel Tank Incl.	20,000
P4	Centrifuge Feed Pump 5 GPM 25'	400
P6	Cooling Water Pump 50 GPM 50'	1,000
S	Fume Scrubber (Recirculating Hypochlorite)	1,500
		109,100
	Contingency Equipment	10,900
	Total Purchased Equipment	120,000
	Delivery and inspection (6%)	7,000
	TOTAL PURCHASED EQUIPMENT COST	$127,000

Table 32

ESTIMATED EQUIPMENT COST FOR A 450 TPY SPRAY DRIED OFC PLANT

T1	Holding Tank - 750 Gal. C.S.	1,000
P1	Centrifuge Feed Pump 17 GPM 25'	500
C1	Dewatering Centrifuge Pfaudler Z2L	23,000
T_2T_3	Holding Tanks 300 Gals. C.S.	1,200
P_2	Moyno Positive Displacement Pump 5 GPM	1,000
P_3	Centrifugal Pump 12 GPM - 75'	500
HE-1	Heat Exchanger 20 sq. ft. Plate on Equip. with controls	2,000
A	SO_2 Addition System - with controls	3,000
$R.R_2$	Reactors 200 psi 400 Gals. 316 SS	5,000
FE	Flash Evaporator Shell	2,000
T4	Holding Tank 500 Gals. 316 SS	1,000
C_2	Continuous Solid Discharge Centrifuge	16,000
T5	Holding Tank 500 Gals. 316 SS	1,000
E	Concentrating Evaporator	8,000
S	Spray Dryer	45,000
Steam	Boiler Packaged Unit 10,000 #/Hr. 150 psi with instrumentation. Fuel Tank Incl.	20,000
P4	Centrifuge Feed Pump 5 GPM 25'	400
P6	Cooling Water Pump 50 GPM 50'	1,000
S	Fume Scrubber (Recirculating Hypochlorite)	1,500
		133,100
	Contingency Equipment	11,900
	Total Purchased Equipment	145,000
	Delivery and inspection (6%)	9,000
	TOTAL PURCHASED EQUIPMENT COST	$154,000

Table 33

ESTIMATED CAPITAL INVESTMENT FOR A 900 TYP - 50% OFC PLANT

Major Equipment		
- as described on Equipment List	$127,000	
		% of Equipment Cost
Other		
- installation	$ 13,000	10%
- piping	$ 13,000	10%
- electrical	$ 10,000	8%
- insulation	$ 5,000	4%
- steam tracing	$ 5,000	4%
- additional instrumentation	$ 5,000	4%
- yard improvements	-	-
	$ 51,000	
Physical Plant Cost		$178,000
		% of Physical Plant Cost
Engineering and Construction	$ 30,000	17.5
Direct Plant Cost		$208,000
		% of Direct Plant Cost
Contingency	$ 15,000	7.5
TOTAL INVESTMENT		$223,000

Table 34

ESTIMATED CAPITAL INVESTMENT FOR A 450 TPY - SPRAY DRIED OFC PLANT

Major Equipment		
- as described on Equipment List	$154,000	
Other		% of Equipment Cost
- installation	$ 15,500	10%
- piping	$ 15,500	10%
- electrical	$ 12,000	8%
- insulation	$ 6,500	4%
- steam tracing	$ 6,500	4%
- additional instrumentation	$ 6,000	4%
- yard improvements	-	-
	$ 62,000	
Physical Plant Cost		$216,000
		% of Physical Plant Cost
Engineering and Construction	$ 38,000	17.5
Direct Plant Cost		$254,000
		% of Direct Plant Cost
Contingency	$ 19,000	7.5
TOTAL INVESTMENT		$273,000

Table 35

ESTIMATED MANUFACTURING COST FOR A 900 TPY - 50% OFC

Product: 50% OFC
Capacity: 900 Tons per year

	$ per year
Raw Materials	
Activated Sludge	--
Sulfur Dioxide (90,000 lbs/year @ 15¢/lb)	13,500
Labor	
Operating	42,000
Maintenance	8,000
Utilities	
Electricity, 60 Kwh @ 1.5¢/Kwh	7,000
Steam 3000 #/Hr. @ $.60/1000	14,500
Supplies	
Spare Parts - Misc.	4,000
DIRECT PRODUCTION COST	89,000
Depreciation, 12 year, straight line	19,000
Insurance 1% Capital Cost	2,000
FIXED CHARGES	21,000
TOTAL MANUFACTURING COST	110,000
Production Cost:	$ 122 per ton $ 0.061 per lb

Table 36

ESTIMATED MANUFACTURING COSTS FOR A 450 TPY - SPRAY DRIED OFC

Product: Spray Dried OFC
Capacity: 450 Tons per year

	$ per year
Raw Materials	
Activated Sludge	--
Sulfur Dioxide (90,000 lbs/year @ 15¢/lb)	13,500
Labor	
Operating	42,000
Maintenance	8,000
Utilities	
Electricity, 70 Kwh @ 1.5¢/Kwh	8,000
Steam 3500 #/Hr. @ $.60/1000	17,000
Supplies	
Spare Parts - Misc.	4,000
DIRECT PRODUCTION COST	92,500
Depreciation, 12 year, straight line	23,000
Insurance 1% Capital Cost	3,000
FIXED CHARGES	26,000
TOTAL MANUFACTURING COST	118,500
Production Cost:	$ 264 per ton $ 0.132 per lb

Further Work

While feasible conditions have been demonstrated in the pilot plant for all the process steps, these conditions are not necessarily optimum. Therefore, further work is needed to demonstrate that more favorable economics are attainable and to provide more precise design information. The need for much of this work is alluded to in the previous discussion section of pilot plant results while some of the work relates to separate economic evaluation as would apply in integration of a large OFC plant with an operating sewage treatment plant.

Process Improvement

- Settling

Use of improved settling equipment or clarifiers should be demonstrated as discussed

- SO_2 Addition

The ease with which SO_2 liquifies, limits the transfer pressure by which liquid SO_2 can be injected against a positive head. The point and rate of SO_2 addition can thus be varied to avoid or minimize this constraint

- Reactor Heating

This central heat transfer problem should be further investigated under both actual operating test conditions, as discussed, and by theoretical studies of alternatives such as steam injection, flue gas utilization.

- Heavy Metal Removal

It remains to be seen if heavy metals concentrations are objectionable in residential area wastewaters. In the general case, however, it is useful to demonstrate that a positive quality control of metals concentrations can be attained in wastewaters derived from both industrial and residential wastes.

- Centrifugation (Separation)

The centrifuge equipment selection basis has been previously discussed. It is possible that vacuum filtration would also prove suitable as a less costly alternative.

This will depend upon a more detailed understanding of the filtering characteristics of the dewatered sludge steam.

- Concentration

The concentration of dilute OFC in the batch still proceeded satisfactorily in the pilot plant. It is likely, however, that multiple effect evaporation will prove more economical, depending upon final product specifications. This alternative, because of its significant cost importance should be evaluated in pilot plant and in technical/economic trade off studies because of its potential cost reduction.

- Corrosion Studies

Pilot plant work was conducted in stainless steel equipment and no corrosion was noted. It is likely that other less costly materials can be used and these alternatives should be evaluated.

Related Economic Data

- Sewage Treatment Plant Cycle Economics

The effect of OFC production is to remove BOD from the plant. While no cost credits have been taken for sludge removal in present cost statements, the design and economic benefits of integrated OFC, and secondary treatment plants should be understood. Similiarly, the portion of the sludge which is not solubilized but is easier to filter to a higher dryness offers additional economic credits.

- Ultimate Waste Disposal

The disposal of sludges by incineration poses complex design alternatives, among them, sludge dryness and heating value. Evaluation of these parameters may improve or adversely affect the attractiveness of hydrolyzed sludge incineration. Where ultimate disposal is sanitary landfill, volume reduction and improved dryness are factors to be considered along with distribution (transportation) costs and augmented landfill site capacity.

- Heat Economy

Waste heat boiler utilization, flue gas enthalpy and digester gas all should be considered in relation to energy requirement to the SO_2 hydrolysis process. These may be particularly important in a retrofit situation in which this process can increase the plant capacity of an older, overloaded plant having these energy sources available.

Next Steps

The conversion of activated sludge, using the SO_2 conditioning process, into an organic feed concentrate (OFC) with nutritional value, has been demonstrated in the laboratory, in a pilot plant trial and in feeding studies.

These studies have shown that this process offers a viable approach to solving the dewatering problem of activated sludge as well as providing an income by selling the OFC as animal feed.

Further activities are needed with two basic objectives:

- Commercialize the process
- Determine the full potential of this process

In order to achieve the first objective, a demonstration pilot plant should be constructed and operated at an operating sewage facility. Additional basic research is necessary to achieve the second objective.

The program we propose for each case is described below

Demonstration - Pilot Plant Operation

It is our judgment that the most successful approach to commercialize this process is by adopting it as part of the normal sewage plant operation. Under these conditions, the process can be evaluated arriving at realistic cost data and the results can be communicated objectively to concerned municipal and engineering groups.

Further, such a demonstration unit can only be effected by the combined efforts of a multidisciplinary team involving municipal management experts, sanitary engineers, chemical engineers, water pollution control plant operating engineers, analytical chemists, biologists, nutritionists, toxicologists, and process control specialists working together under a single unit which can tie these talents together and apply them in a municipally-oriented problem and environment.

The project should include:

- Design and construction of a demonstration facility
- Verification of the process in an actual plant
- Development of process control requirements
- Optimization of process
- Evaluation of end products, including OFC and the solids fractions

In addition, this demonstration unit will serve as the pilot plant to study factors relative to the process which have not been studied before. These include:

- Prethickening procedures
- Method of sulfur dioxide addition
- Pressure/temperature mode of operation such as steam injection versus jacketed vessel with agitation
- Alternate method of separating OFC and insoluble solids
- Treatment of OFC for heavy metal control by various methods, such as liming, peroxide, etc.
- Methods of concentration or dehydration of both soluble and insoluble streams.

It is important to note, that the end products received from these plants, should be evaluated both chemically, and biologically for their nutritional value and toxicological effects.

Basic Research

This proposed program can be divided into two major areas which are described herein.

(1) Suitability of process for mixed sludge:
Since mixed sludge is composed of raw and activated sludge, it may be possible to convert the solids into a feed concentrate via the sulfur

dioxide conditioning route. Initial studies in our laboratory showed solids conversion of about 30% but no evaluation of the resulting feed concentrate was carried out.

Based on theoretical considerations, this approach seems feasible, indeed. However, in order to increase the conversion and food value, higher concentrations of sulfur dioxide may be required. This is becaused the hydrolytic effect of sulfur dioxide should be emphasized in this case, whereas, in activated sludge cell wall rupture is the major concern.

If sucessful, this investigation will widen the scope of this invention. Two advantages may result:

- elimination of anaerobic digestion
- reduction of the cost of OFC

(2) Utilization of the insoluble fraction

The non-soluble fraction, obtained after SO_2 conditioning, is believed to consist mainly of high molecular weight polysaccharides which are part of bacterial cell wall. To date, no nutritional or chemical data are available on this fraction. In order to assess the value of this fraction it is suggested that:

- the possibility of breaking down these chains further with SO_2, using high concentration, be evaluated
- the nutritional value of the solubilized materials be determined
- the possibility of solubilizing these materials using enzymes such as lysozymes or mixture of other enzymes be investigated. While enzymatic treatment may seem an expensive operation at first, the development of enzyme immobilization techniques, allowing reuse of enzymatic preparations, may lower the cost

Section V

SAFETY EVALUATION OF OFC

MATERIALS AND METHODS

Pilot Study - Determination of Feeding Levels

Objective of Pilot Study

A pilot experiment was performed to determine the acceptability to rats of prepared diets containing a dry, finely-pulverized, SO_2-treated-activated-sludge (Organic Feed Concentrate; OFC) present in varying concentrations prior to the initiation of the toxicity evaluation (reproduction/teratology study) in rats and rabbits.

Procedure

Seven experimental diets were prepared. Variation between diets was limited to:

- total carbohydrate contribution
- type of carbohydrate added
- content of pulverized, dry, OFC

Diets containing 68% carbohydrate, either as starch (I) or an equimixture of starch and sucrose (II) were included as control diets for the experiment. Dry OFC was added to the remaining diets (III, IV, V, VI, VII) and an equivalent amount of carbohydrate was removed. Components and percentage composition of each diet is shown in Table 37. A total of 200 grams of each diet was prepared. Mineral content of the OFC-containing diet was not adjusted to compensate for the ash content of the sludge.

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Table 37

PERCENTAGE COMPOSITION OF EXPERIMENTAL DIETS

	------ Experimental Diet Groups ------						
Component	I	II	III	IV	V	VI	VII
Casein	15	15	15	15	15	15	15
Cottonseed Oil	6	6	6	6	6	6	6
Vitamin Mix*	1	1	1	1	1	1	1
Salt Mix**	4	5	4	4	4	4	4
Cellulose	1	1	1	1	1	1	1
Water	5	5	5	5	5	5	5
Corn Starch	68	34	63	58	29	53	48
Sucrose	-	34	-	-	29	-	-
Treated Sludge	-	-	5	10	10	15	20
Total (%)	100	100	100	100	100	100	100

* The vitamin mix (A.O.A.C.) added at 1% of the diet provided (in mg/kg of diet): vitamin A, 39.6825; vitamin D, 40492, alpha tocapherol acetate, 485.0090; ascorbic acid, 1017.5200; 1-inositol, 110.2290, choline dihydrogen citrate, 3715.1230; menadione, 49.6030; p-aminobenzoic acid, 110.2290; niacin, 99.2060; riboflavin, 22.0450, pyridoxine HCL, 22.0450; thiamine HCL 22.0450; calcium pantothenate, 66.1376; biotin, 0.44092; folic acid, 1.98412; vitamin B_{12}, 29.7619.

** The mineral mix (U.S.P. XVII) added at 4.0% of the diet contained (in percent composition): calcium carbonate, 38.140; cobalt chloride, 0.0023; cupric sulfate, 0.0477; ferrous sulfate, 2.700; magnesium sulfate, 5.730; manganese sulfate, 0.401; potassium iodide, 0.079; potassium phosphate monobasic, 38.900; sodium chloride, 13.930; zinc sulfate, 0.0548.

The OFC utilized in the experiment was a composite of laboratory-scale preparations. However, it was assumed that this material would be representative of the material to be produced on a pilot-plant scale.

Fourteen immature Sprague-Dawley derived rats (7M: 7F) with an average body weight of 69 grams (range 55-90 grams) were used in the experiment. One male and one female, individually housed, were fed (ad libitum) one of the seven diets over a one-week period. Rats were weighed immediately prior to placement of the assigned diet in the cage and again at the conclusion of the seven-day feeding period. Food consumption for each animal was measured three times during the study (days 2, 4 and 7) and the total (seven-day) consumption was recorded. Animals were observed daily for normal or abnormal activity, acceptablility of the diet and fecal character.

Results

No clear-cut differences in acceptability of each of the diets was apparent. However, only animals on those diet into which sucrose had been incorporated showed any measurable weight-gain. A tabulation of body-weight and food-consumption data is shown in Table 38.

Table 38

BODY WEIGHT AND FOOD CONSUMPTION DATA

		Body Weight (grams)			Food Consumption	
Diet	Sex	Initial	Final	% Change	Grams 7-Day	% of offered
I	M	90	70	-22	75	75
	F	70	60	-14	76	76
II	M	70	80	+14	96	96
	F	55	86	+56	73	73
III	M	70	63	-10	93	93
	F	70	60	-14	93	93
IV	M	65	62	-5	90	90
	F	70	57	-19	94	94
V	M	75	84	+11	81	81
	F	65	73	+11	81	81
VI	M	60	57	-5	100	100
	F	70	65	-7	85	85
VII	M	65	59	-9	79	79
	F	70	62	-11	64	64

Although the total consumption of the two control diets (I, II) was comparable, a weight-gain was seen in those animals fed the sucrose-containing diet (II). Of further interest is the somewhat increased food intake (compared to controls) in four of the five OFC containing diets. However, in this brief experiment with a limited number of animals, the significance of this observation could not be determined. In the groups fed the OFC, an increase in body weight was seen only in those rats receiving sucrose as a part of the diet (V).

No significant change in appearance or general health of the animals was noted. Fecal quantity and texture was normal. The coloration of the fecal pellets was somewhat lighter than that excreted by rats fed a standard laboratory chow.

Conclusions

From observations made during this pilot experiment, the following conclusions were drawn:

- The incorporation of as much as 20% dry OFC into prepared rat diets did not appear to noticeably alter the consumption of the diet.
- No acceptability differences related to OFC concentrations were apparent.
- The inclusion of some sucrose as a part of the carbohydrate contribution of the diet appeared to influence the growth of the rats under the condition of the study.

Diet Preparations

Approximately 170 pounds of flowable, powdered, OFC was obtained from activated sludge. The product was prepared for toxicological evaluation in the three laboratory species - rats, rabbits and chickens. The powdered OFC was blended to a homogeneous mixture and sampled for proximate analysis and amino acid profile prior to incorporation into any of the experimental diets.

Proximate Analysis and Amino Acid Profile

The proximate composition of the OFC is shown in Table 39.

Table 39

PROXIMATE COMPOSITION OF SPRAY DRIED OFC

Component	Percent
Moisture	8.37
Nitrogen	10.0
Total Lipid	1.90
Ash	13.78
Fiber	1.25
Carbohydrate (by difference)	12.20

The amino acid profile determined on a 16-hour hydrolysate prepared with constant boiling HC1 is summarized in Table 40.

Table 40

AMINO ACID PROFILE OF SPRAY DRIED OFC

Amino Acid	Percent
Lysine	1.58
Histidine	0.49
Arginine	0.86
Aspartic Acid	1.77
Threonine	1.16
Serine	1.04
Glutamic Acid	3.22
Proline	1.16
Glycine	1.77
Alanine	2.44
Cystine	0
Valine	1.61
Methionine	0.41
Isoleucine	1.04
Leucine	1.87
Tryosine	0.78
Phenylalanine	1.05
Tryptophan	0.21
Total	22.46

Based upon the conclusions distilled from the pilot study, rat diets modeled after those used in the study were prepared for the rat mating, reproduction and teratology evaluation. Composition of the experimental diets is shown in Table 41.

Table 41

COMPOSITION OF DIETS USED IN RAT MATING AND REPRODUCTION STUDY

Component	I	II	III
Casein	18	18	18
Cottonseed Oil	5	5	5
Vitamin Mix	1	1	1
Salt Mix	5	3.4	1.8
Cellulose	1	1	1
Water	5	5	5
Corn Starch	35	26.6	18.2
Sucrose	30	30	30
Treated Sludge	0	10	20
Total (%)	100	100	100

Composition of the vitamin and salt mixes was identical to that used in the pilot study. The casein content was increased slightly. Salt mix content of the OFC containing diets was adjusted, taking into account the ash content of the OFC.

Evaluation of the effects of two dietary levels of OFC, 10 and 20 percent, was planned. Weanling male rats, with a body-weight range of from 25 to 45 grams were placed on the diets. During the initial 48 hours of the study, food intake of rats in both control and experimental groups was negligible and several animals in each of the groups died. Consequently, surviving animals were returned to a standard laboratory ration for a three-week period. Animals that died were replaced and the feed program was again started.

Growth pattern and food consumption observed during the first two weeks of the study are presented in Table 42.

Table 42

GROWTH PATTERN AND FOOD CONSUMPTION OF RATS AFTER TWO WEEKS FEEDING

Group	Dietary Sludge Level (%)	Avg. Body Wt. (gm) Initial	1-wk	2-wk	Food Consumption (gm/day/rat) Week 1	Week 2
I	0	117	133	150	11.6	15.6
II	10	124	126	141	9.3	14.0
III	20	130	113	114	5.8	6.1

During the second week of feeding, pronounced diarrhea was seen in nearly all animals at the 20% OFC level. Stools were black in color and were viscous. Two rats in the group failed to survive the two-week period. No gross lesions were noted at autopsy of these animals.

Food consumption and weight-gain of animals at the 10% OFC level approached that seen in control animals after two weeks of feeding.

Since it was obvious that continued feeding of the 20% OFC diet would probably result in death to all animals in the group, feeding of the group was stopped and a replacement group III at a 5% OFC level was started.

Table 43 presents the diet composition of the replacement group III.

Table 43

COMPOSITION OF REPLACEMENT GROUP III DIET USED IN RAT MATING AND REPRODUCTION STUDY

Component	Group III Percentage Composition
Casein	18
Cottonseed Oil	5
Vitamin Mix	1
Salt Mix	4.2
Cellulose	1
Water	5
Corn Starch	30.8
Sucrose	30
Treated Sludge	5

Based on these feeding results, all rat diets used for mating and reproduciton (Phase I), teratology (Phase II) and neonatal effects (Phase III) studies utilized the 5 and 10% OFC levels.

Rabbit diets for teratology study were similarly constructed to supply 5 and 10% OFC levels to test animals. The composition of diets used in the rabbit teratology study is presented in Table 44.

Table 44

COMPOSITION OF DIETS USED IN RABBIT TERATOLOGY STUDY

Component	Percentage Composition I	II	III
Casein	25	25	25
Corn Starch	20	11.6	15.8
Glucose H_2O	10	10	10
Cellulose	15	15	15
Cottonseed Oil	10	10	10
Vitamin Mix	1	1	1
Salt Mix	6	4.4	5.2
Arginine	0.3	0.3	0.3
Ascorbic Acid	0.2	0.2	0.2
Sucrose	12.5	12.5	12.5
OFC	0	10	5
Total (%)	100	100	100

In determining the egg-production in chickens, four test levels were utililized. The diets used are presented in Table 45.

Table 45

COMPOSITION OF DIETS USED IN CHICKEN EGG PRODUCTION STUDY

Component	Percentage Composition I	II	III	IV	V
Soy Assay Protein	30	30	30	30	30
Glucose H_2O	20	20	20	20	20
Sucrose	26.07	21.87	17.67	13.47	9.27
Corn Oil	16	16	16	16	16
Salt Mix	6	5.2	4.4	3.6	2.8
Glycine	1	1	1	1	1
Methionine	0.6	0.6	0.6	0.6	0.6
Vitamin A	0.1	0.1	0.1	0.1	0.1
Vitamin D_3	0.03	0.03	0.03	0.03	0.03
Vitamin Mix	0.2	0.2	0.2	0.2	0.2
OFC	0	5	10	15	20
Total (%)	100	100	100	100	100

A commerical laying diet (Purina^R Layena^R (N) CF) was used as control on the semi-synthetic diet constructed for the laying experiment. OFC at 5, 10, 15 and 20% levels were incorporated into this diet on a weight basis for additional study. The guaranteed analysis and ingredients of this diet prior to incorporation of the sludge is shown in Table 46.

Table 46

GUARANTEED ANALYSIS AND INGREDIENTS OF PURINA (R) LAYENA (R) (N) CF[1]

Guaranteed Analysis

Crude protein not less than	16.0%
Crude fat not less than	3.0%
Crude fiber not more than	4.5%
Calcium (Ca) not less than	2.8%
Calcium (Ca) not more than	3.8%
Phosphorus (P) not less than	0.6%
Iodine (I) not less than	0.00008%
Salt (NaCl) not less than	0.3%
Salt (NaCl) not more than	0.9%

Ingredients

Ground yellow corn, soybean meal, meat and bone meal, dehydrated alfalfa meal, brewers' dried grains, wheat middlings, cane molasses, vitamin B_{12} supplement, vitamin A supplement, BHT (a preservative) methionine hydroxy analogue calcium, choline chloride, calcium pantothenate, niacin, riboflavin supplement, D activated animal sterol, folic acid, menadione sodium bisulfite (source of vitamin K activity), low fluorine rock phosphate, calcium carbonate, iodized salt, manganous oxide, copper sulfate, zinc oxide.

(1) Ralston Purina Company, St. Louis, Missouri

Reproduction Studies in Three Species

Three studies in rats, one study in rabbits and one study in chickens were performed to determine the potential effects of the organic food concentrate derived from sewerage sludge on various reproductive parameters. Phase I studies, performed in rats were designed to show the effect, if any, on mating behavior, fertility and nidation. Phase II studies, using rats and rabbits determined terotogenic potential. Parturition and neonatal development were studied in rats in Phase III of this investigation. Chickens were utilized to determine the effect of OFC on egg-laying production.

PHASE I - Fertility and Reproduction Performance in Rats

Three groups of 20 weanling male rats (Sprague-Dawley) and three groups of 20 young adult female rats (Sprague-Dawley) were used in this phase of the study. Group sizes were selected to permit the generation of 18-20 litters per treatment group. Animal identification and group assignment are presented in Table 47.

Table 47

GROUP ASSIGNMENT AND ANIMAL IDENTIFICATION, PHASE I - RATS

Group	Material	Animal # and Sex	Duration of Feeding Prior to Mating	After Mating
I	Control	1-20 Male	6 weeks	-----
		2,4,7,9,11,14,17, 18,19,20 Female	2 weeks	13 days
		1,3,5,6,8,10,12, 13,15,16 Female	2 weeks	To weaning
II	10% OFC	21-40 Male	6 weeks	------
		21,22,25,27,28,32 33,34,37,38 Female	2 weeks	13 days
		23,24,26,29,30,31, 35,36,39,40 Female	2 weeks	To weaning
III	5% OFC	41-60 Male	6 weeks	-----
		41,42,45,46,47,49, 50,56,57,60 Female	2 weeks	13 days
		43,44,48,51,52,53, 54,55,58,59 Female	2 weeks	To weaning

During the pre-mating dosing period animals were caged individually in temperature and humidity controlled quarters.

Male rats were fed a control diet, or diets containing 5 or 10% OFC ad lib for 6 weeks prior to mating and for 4 additional weeks during the mating process. Weekly body weights and food consumption were recorded during the pre-mating period to determine additional effects on diet acceptability and animal growth.

Ad lib feeding of diets to the females which were permitted to cast their litters naturally was initiated 14 days prior to mating and continued through gestation day 5. Body weights and food consumption were monitored during the 2-week pre-mating feeding period. Female rats scheduled for sacrifice on the 13th day of gestation (day 14) were fed the test diets to the time of sacrifice.

On the assigned mating day, males and females were caged together (1M and 1F). The selection of animals for mating was made randomly within dosing groups. Animals were housed together until there were obvious signs of successful mating (mucous plug; sperm in vaginal smear) at which time the sexes were separated. Females were then caged individually and the males were sacrificed.

All animals were checked daily for survival, behavior and appearance.

Approximately ten females from each treatment group were permitted to cast litters naturally. Pup count and weight were recorded at parturition and on the 4th post-partum day. Litter-size was then reduced to a maximum of 8 pups which were observed through weaning (21 days). Pup count and weight was again recorded at this time.

The effects of the test formulations on fertility, gestation and lactation performance were evaluated by comparison of the following indexes:

- Fertility Index - percent of mating resulting in pregnancies
- Gestation Index - percent of pregnancies resulting in litters cast alive
- Viability Index - percent of pups cast alive surviving four days
- Lactation Index - percent of pups alive at 4 days (after culling of litters) surviving to weaning (21 days)

Ten females from each of the treatment groups were sacrificed on the calculated 13th gestation day. The ovaries, uterus and fetuses of each animal were examined and the number of corpora lutea, implantation sites and resorption sites were recorded. In addition, the parental females and fetuses were examined for any apparent abnormalities.

The testes from all parental males were preserved in 10% neutral formalin for possible histopathologic examination.

PHASE II - Teratology in Rats

Sixty young adult female rats (Sprague-Dawley) inseminated at these laboratories were used in this Phase II study. Animal identification and group assignment are presented in Table 48.

Table 48

GROUP ASSIGNMENT AND ANIMAL IDENTIFICATION, PHASE II - RATS

Group	Material	Animal # (Females)
I	Control	1-20
II	10% OFC	21-40
III	5% OFC	41-60

Each of the animals was individually housed and observed daily for survival, behavior and general appearance. Individual body weights were obtained on the 19th day of gestation.

All animals were fed the control diet until the 5th gestation day. Animals in groups II and III were then fed diets and *ad lib* incorporating the test material at 10 and 5% respectively. The control and test animals were continued on their respective diets until the 14th day of pregnancy. At this time, test animals were returned to the control diet. Feeding then continued until the 19th gestation day.

On the calculated 19th day of gestation, parental females were weighed, anesthetized and a laparotomy was performed on each animal. The ovaries and uteri of each female were examined for any obvious abnormalities. The corpora lutea, implant sites and resorption sites were counted and any evidence of abnormal *in utero* development was recorded.

The litters were removed and examined. Viable and non-viable fetuses were counted, weighed and examined grossly. Live fetuses were sacrificed following a four hour incubation period in a temperature and humidity controlled incubator (38°C and 55% humidity).

Approximately two-thirds of the fetuses were cleared for skeletal staining by the potassium hydroxide/glycerine technique and the skeletons were stained with Alizaren-Red S. The soft tissues of the remaining one-third of the fetuses were examined grossly after formalin-fixation.

PHASE II - Teratology in Rabbits

Thirty adult female (New Zealand White) rabbits artificially inseminated in these laboratories were to be used in this study. However, the absence of pregnancies among these animals made it necessary to use a second group of does (New Zealand White) again inseminated at the Biological Science Laboratories in order to obtain approximately 10 pregnant females per treatment group. Since insemination error was responsible for the lack of success in the first group of animals utilized for this study, they were eliminated from consideration and will not be discussed. Animal identification and group assignment for the 30 rabbits ultimately used appears in Table 49.

Table 49

GROUP ASSIGNMENT AND ANIMAL IDENTIFICATION, PHASE II - RABBITS

Group	Material	Animal # (Female)
I	Control	1-10
II	10% OFC	11-20
III	5% OFC	21-30

Each of the animals was individually housed and observed daily for survival, behavior and general appearance. Individual body weights were obtained on the 29th day of gestation.

All animals were fed the control diet until the 6th gestation day. Animals in groups II and III were then fed diets ad lib with OFC levels of 10 and 5% respectively. The control and test animals were continued on their respective diets until the 18th day of pregnancy. At this time, test animals were returned to the control diet. Feeding then continued until the 29th gestation day.

Each of the animals was observed daily for survival, behavior and general appearance. Individual body weights were obtained on the 5th and 29th day of gestation.

On the calculated 29th day of gestation, parental females were weighed, anesthetized and a laparotomy was performed on each animal. The ovaries and uterine horns of each female were examined for any obvious abnormalities. The corpora lutea, implant sites and resorption sites were counted and any evidence of abnormal in utero development was recorded.

The fetuses were removed and examined. Viable and nonviable fetuses were counted, weighed and examined grossly. The viable fetuses from the control and each treatment group were placed in an incubator for observation of 18-hour survival. Following the determination of 18-hour incubation survival, all viable fetuses were sacrificed. Approximately two-thirds of the fetuses were cleared for staining by the potassium hydroxide/glycerine technique and the skeletons were stained with Alizaren-Red S. The soft tissues of the remaining one-third of the fetuses were examined grossly after formalin-fixation and dissection.

Gross examination of the fetuses included observation of:

- the general development
- the cranial contour
- the limb and digital development
- the trunk area

Examination of stained fetuses provided a much more detailed study of the skull, axial skeleton, long bone and appendage development.

Formalin-fixed fetuses were dissected and a thorough examination of the viscera was performed to ascertain the presence of any abnormal development of visceral structures.

PHASE III - Perinatal and Postnatal Study in Rats

Three groups of 20 young adult female rats each (Sprague-Dawley) were used in the study. Stock colony Sprague-Dawley male rats were housed (1M and 1F) together with test females until there were obvious signs of successful mating (mucous plug and sperm in vaginal smear) at which time the sexes were separated. Females were individually caged and the males were returned to the stock colony. Animal identification and group assignment is presented in Table 50.

Table 50

GROUP ASSIGNMENT AND ANIMAL IDENTIFICATION, PHASE III - Rats

Group	Material	Animal # (Female)
I	Control	1-20
II	10% OFC	21-40
III	5% OFC	41-60

Animals were checked daily for survival, behavior and appearance.

All rats were fed the control diet until the 15th day of gestation. Group II and III animals were fed diets containing 10% and 5% OFC respectively ad lib from the 15th gestation day through parturition and the entire lactation period until pups were weaned.

Pup count and weight were recorded at parturition and on the 4th post-partum day. The size of each litter was then reduced to a maximum of eight pups which were observed through weaning (21 days). Sacrificed pups were examined grossly for the presence of any anomalies. Pup count and weight were recorded again at weaning (21 days).

The effects of the test formulations on fertility, gestation and lactation performance were evaluated by comparison of the following indexes:

- Fertility Index - percent of mating resulting in pregnancies
- Gestation Index - percent of pregnancies resulting in litters cast alive
- Viability Index - percent of pups cast alive surviving four days
- Lactation Index - percent of pups alive at four days (after culling of litters) surviving to weaning (21 days)

Egg Production in Chickens

The egg-laying performance of active laying chickens (White Leghorn) fed a synthetic diet incorporating varying concentrations of OFC was monitored through an initial 6-week experimental period. Through this phase of the study each group consisted of nine birds. Group identification and dietary assignment are presented in Table 51.

Table 51

GROUP IDENTIFICATION AND DIETARY ASSIGNMENT FOR CHICKENS IN EGG PRODUCTION STUDY

Group	Dietary Level	Number of Chickens
I	Control	9
II	5% OFC	9
III	10% OFC	9
IV	15% OFC	9
V	20% OFC	9

All animals were fed the control diet for one month prior to assignment to one of the experimental groups. Daily egg production was monitored, by group, and an average normal daily egg production figure was arrived at for each set of nine birds.

At the conclusion of this phase of the study, i.e. feeding OFC in synthetic diet, two birds from each group were sacrificed and gross necropsies were performed.

To determine the reversibility of the effect on egg-production, all remaining birds were placed on a commercial diet (Purina$^{(R)}$ Layena$^{(R)}$ (N) CF) for a four week period. Daily egg production records were kept during this period.

As a control of the synthetic diet test a second series of OFC diets were fed to the remaining chickens, utilizing the commercial diet. The same animals were returned to the same feeding levels previously used in the synthetic diet study: 0, 5, 10, 15 and 20% OFC incorporated by weight into the commercial chow. Daily egg production records were kept per 7 birds for the four week feeding period.

The Effect of OFC in Chicken Diets on the Taste of Eggs as Measured by the Sensory Panel Technique

1. Methodology

(a) Triangle Tests

The triangle method is the most sensitive technique for judging differences between samples. The procedure consists of presenting the judges simultaneously with a set of three samples, two of which are identical, and asking them to identify the odd sample. The judges are also asked to comment on how the odd sample differs from the others. A typical triangle test score sheet is shown in Table 116.

2. Selection of Judges

Ten trained judges, who had previously participated in similar tests, were selected from the sensory panel reserve pool.

3. Test Conditions

The tests were conducted in an odor-free, air conditioned room used exclusively for sensory panel tests. Each judge was seated in an individual booth to prevent contact between judges during testing. Talking was prohibited.

4. Sample Preparation Presentation

On June 19, 1972, eight eggs were submitted as follows:

4	-	Controls	2 dated 6/15/72 2 dated 6/16/72
4	-	OFC fed two weeks	2 dated 6/15/72 2 dated 6/16/72

On June 29, 1972, eight eggs were submitted as follows:

4	-	Controls	1 dated 6/21/72 2 dated 6/22/72 1 dated 6/23/72
4	-	OFC Fed four weeks	1 dated 6/20/72 2 dated 6/22/72 1 dated 6/23/72

For each test the four eggs were broken and combined in a stainless steel bowl. They were beaten with a hand beater for 30 seconds, transferred to a Teflon lined pan coated with 2 g of butter, and scrambled for 3 minutes with constant stirring. Small portions were served hot to the judges in waxlined paper cups identified only by random double number codes.

An additional evaluation was made August 23, 1972 of eggs layed by chickens fed OFC for 4 weeks. Taste evaluation was made as describe above.

RESULTS AND DISCUSSION

PHASE I - Fertility and Reproduction Performance in Rats

The effects of dietary administration of OFC levels of 5 and 10% were evaluated by comparison of several parameters determined for treatment and control groups. These parameters included:

- male and female weight gain and food consumption prior to mating
- male and female fertility
- incidence of live and stillbirths, size and weight of litters cast naturally
- pup survival and growth
- incidence of corpora lutea, implant sites, resorption sites and fetal abnormalities in pups delivered by ceasarian section

Parental Survival and Growth

All male and female animals survived the Phase I reproduction study. Male and female animals receiving 5% OFC in the diet consistently outperformed control animals as to rate of growth and daily food consumption. Animals receiving the 10% OFC diets, however, consumed significantly less diet and therefore grew at a slower rate than the controls. This was true, more so for the females than the males. Average body weights are presented in Table 52.

Table 52

AVERAGE BODY WEIGHTS (GRAMS) - PHASE I RATS

		Study Week							
Group	Sex	0	1	2	3	4	5	6	Net Change
I	M	117	133	150	169	195	215	243	+126
Control	F	-	-	-	-	206	214	223	+15
II	M	124	126	141	139	163	187	203	+79
10% OFC	F	-	-	-	-	195	195	196	+1
III	M	128	168	192	232	262	293	318	+190
5% OFC	F	-	-	-	-	194	203	215	+21

Table 53 presents the average food consumption for these animals.

Table 53

AVERAGE FOOD CONSUMPTION - PHASE I RATS

		Study Week					
Group	Sex	1	2	3	4	5	6
I	M Mean (g)	81	109	124	121	112	124
	g/day	11.6	15.6	17.7	17.3	16.0	17.7
	F Mean (g)					78	86
	g/day					11.1	12.3
II							
10% OFC	M Mean (g)	65	98	95	107	106	111
	g/day	9.3	14.0	13.6	15.3	15.1	16.0
	F Mean (g)					53	52
	g/day					7.6	7.4
III							
5% OFC	M Mean (g)	82	113	127	149	148	158
	g/day	11.7	16.1	18.1	21.3	21.1	22.6
	F Mean (g)					95	95
	g/day					13.6	13.6

In Tables 54 through 59 the individual body weight data are presented and Tables 60 through 65 give the individual food consumption data for these animals.

Table 54

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Individual Body Weights (grams)

Group: I Control
Sex Male

Animal Number	Study Week							Net Change
	0	1	2	3	4	5	6	
1	91	99	125	143	142	180	197	+ 106
2	113	108	150	192	228	245	278	+ 165
3	147	164	195	248	273	278	300	+ 153
4	108	148	155	197	228	245	260	+ 152
5	107	108	130	173	181	172	210	+ 103
6	113	127	115	135	122	157	173	+ 60
7	120	129	145	192	221	240	265	+ 145
8	170	189	183	200	218	193	204	+ 34
9	134	141	160	205	249	255	265	+ 131
10	110	120	107	125	185	205	256	+ 146
11	110	127	147	165	209	228	265	+ 150
12	107	123	141	143	181	210	248	+ 141
13	110	122	140	143	197	231	272	+ 162
14	90	97	125	125	157	190	227	+ 137
15	101	118	130	124	152	198	232	+ 131
16	144	134	160	162	175	202	240	+ 96
17	103	158	170	178	227	240	262	+ 159
18	133	169	185	185	147	183	210	+ 77
19	140	162	235	223	278	287	300	+ 160
20	92	109	103	117	137	168	197	+ 105
Mean	117	133	150	169	195	215	243	+ 126
Range	90- 170	99- 189	103- 235	117- 248	122- 278	157- 287	173- 30	34- 165

Table 55

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Individual Body Weights (grams)

Group: II 10% OFC
Sex Male

Animal Number	Study Week 0	1	2	3	4	5	6	Net Change
21	92	112	128	145	186	215	238	+ 146
22	136	115	135	170	162	200	223	+ 87
23	107	103	97	103	131	160	180	+ 73
24	60	91	110	133	161	175	205	+ 145
25	90	128	150	177	199	215	233	+ 133
26	178	165	193	188	171	198	145	- 33
27	103	98	127	127	175	215	240	+ 137
28	111	107	140	143	156	152	182	+ 71
29	109	101	105	102	142	177	208	+ 99
30	128	126	133	115	104	88	84	- 44
31	120	108	145	135	189	223	250	+ 130
32	128	158	168	154	199	238	263	+ 135
33	167	191	200	176	215	240	258	+ 91
34	177	158	155	140	167	195	216	+ 39
35	130	124	145	138	138	145	150	+ 20
36	104	128	150	125	118	140	159	+ 55
37	145	134	150	103	151	173	192	+ 47
38	128	122	125	154	190	217	234	+ 106
39	134	137	150	121	129	173	190	+ 53
40	128	115	123	138	168	195	225	+ 97
Mean	124	126	141	139	163	187	203	779
Range	60- 178	91- 191	97- 200	102- 188	104- 215	88- 240	84- 263	44- 146

Table 56

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Individual Body Weights (grams)

Group: III 5% OFC
Sex: Male

Animal Number	Study Week 0	1	2	3	4	5	6	Net Change
41	135	185	200	256	282	295	338	+ 203
42	158	180	178	227	260	281	293	+ 135
43	133	182	210	252	290	325	353	+ 220
44	98	143	185	194	230	257	283	+ 185
45	130	176	203	225	274	312	308	+ 178
46	154	190	212	255	268	312	340	+ 186
47	131	160	196	252	294	338	350	+ 229
48	123	150	173	192	200	220	217	+ 94
49	139	163	200	245	262	315	353	+ 214
50	131	177	215	248	308	342	381	+ 250
51	133	185	220	244	242	248	280	+ 147
52	104	162	198	251	290	326	380	+ 276
53	108	166	205	240	275	300	340	+ 232
54	133	173	208	245	260	286	308	+ 175
55	130	158	183	237	280	276	316	+ 186
56	140	167	194	202	223	242	268	+ 128
57	123	185	185	234	275	322	361	+ 238
58	111	128	125	185	230	285	315	+ 204
59	124	147	166	208	243	273	273	+ 149
60	138	185	188	250	258	310	294	+ 156
Mean	128	168	192	232	262	293	318	190
Range	104-158	128-190	125-220	185-256	200-308	220-342	217-381	94-276

Table 57

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Individual Body Weights (grams)

Group: I Control
Sex: Female

Animal Number	Study Week 0	Study Week 1	Study Week 2	Net Change
1	214	222	230	+16
2	220	230	238	+18
3	192	201	207	+15
4	160	171	178	+18
5	178	181	190	+12
6	208	212	222	+14
7	190	200	207	+17
8	200	208	217	+17
9	218	227	239	+21
10	215	220	226	+11
11	254	259	266	+12
12	266	271	280	+14
13	207	214	223	+16
14	190	196	206	+16
15	205	213	222	+17
16	200	207	218	+18
17	186	188	196	+10
18	175	184	195	+20
19	259	263	271	+12
20	217	222	231	+14
Mean	207	214	223	15
Range	266	263	280	21

Table 58

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Individual Body Weights (grams)

Group: II 10% OFC
Sex: Female

Animal Number	--------Study Week-------			Net Change
	0	1	2	
21	155	155	155	0
22	200	201	203	+ 3
23	131	131	130	- 1
24	180	179	178	- 2
25	220	223	222	+ 2
26	210	211	216	+ 6
27	190	189	193	+ 3
28	230	230	232	+ 2
29	250	245	241	+11
30	230	231	231	+ 1
31	200	203	207	- 7
32	165	165	160	- 5
33	200	201	217	+ 7
34	200	204	205	+ 5
35	200	204	211	+11
36	210	211	207	- 3
37	230	225	227	- 3
38	175	165	163	-12
39	175	176	178	+ 3
40	160	162	161	+ 1
Mean	195	195	196	1
Range	131- 250	131- 245	130- 232	12- 11

Table 59

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Individual Body Weights (grams)

Group: III 5% OFC
Sex: Female

Animal Number	--------Study Week------			Net Change
	0	1	2	
41	180	190	202	+22
42	194	205	214	+20
43	170	180	193	+23
44	225	237	255	+30
45	223	230	238	+15
46	200	211	213	+13
47	185	198	207	+22
48	180	195	202	+22
49	213	218	238	+25
50	210	222	228	+18
51	165	176	185	+20
52	180	185	202	+22
53	190	197	203	+13
54	200	208	225	+25
55	200	201	213	+13
56	225	234	247	+22
57	175	182	205	+30
58	195	204	213	+18
59	200	212	233	+33
60	175	188	204	+29
Mean	194	203	215	21
Range	170-	176-	185-	13
	225	237	247	33

Table 60

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Individual Food Consumption (grams)

Group: I Control
Sex: Male

Animal Number	Study Week					
	1	2	3	4	5	6
1	73	112	130	89	105	78
2	81	134	117	124	115	137
3	67	81	120	136	123	87
4	94	87	126	142	112	123
5	78	109	120	87	77	152
6	75	121	90	67	93	91
7	81	116	116	119	116	131
8	74	134	124	107	151	125
9	79	110	144	135	123	152
10	41	74	132	130	116	141
11	79	110	144	135	123	152
12	55	49	110	129	128	140
13	100	110	125	138	122	147
14	99	116	135	96	91	120
15	107	123	117	96	91	120
16	83	72	135	142	130	133
17	108	136	133	146	97	105
18	73	132	109	121	108	129
19	93	115	136	150	108	120
20	83	116	128	119	107	125
Mean	81	109	124	121	112	124
gm/day	11.6	15.6	17.7	17.3	16.0	17.7

Table 61

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Individual Food Consumption (grams)

Group: II 10% OFC
Sex: Male

Animal Number	Study Week 1	2	3	4	5	6
21	57	86	87	121	112	124
22	55	108	99	106	140	116
23	68	75	65	90	90	103
24	53	107	96	143	130	139
25	68	117	96	116	103	111
26	62	86	80	111	113	49
27	61	88	84	120	136	137
28	69	103	97	85	58	107
29	59	69	79	104	103	109
30	57	118	87	73	46	95
31	69	100	123	127	114	122
32	74	110	104	130	123	120
33	61	101	107	140	130	132
34	55	108	115	108	118	101
35	74	102	107	123	99	132
36	64	95	103	47	70	88
37	77	100	94	105	91	102
38	70	110	96	116	123	113
39	72	77	82	76	113	101
40	74	97	108	96	110	119
Mean	65	98	95	107	106	111
gm/day	9.3	14.0	13.6	15.3	15.1	16.0

Table 62

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Individual Food Consumption (grams)

Group: III 5% OFC
Sex: Male

Animal	Study Week					
Number	1	2	3	4	5	6
41	96	118	141	157	132	145
42	77	105	106	152	135	158
43	91	118	132	169	160	151
44	73	103	118	88	147	135
45	85	121	124	219	152	187
46	88	128	131	175	317	167
47	82	111	128	161	167	168
48	76	100	101	98	98	95
49	75	98	126	126	144	157
50	91	125	131	164	160	161
51	83	128	152	156	152	150
52	80	110	146	164	173	171
53	45	87	118	160	150	145
54	92	115	143	139	150	148
55	71	103	126	155	141	195
56	83	121	152	167	163	147
57	59	104	131	159	162	184
58	102	95	100	152	160	165
59	104	144	111	158	135	165
60	93	129	122	152	149	175
Mean	82	113	127	149	148	158
gm/day	11.7	16.1	18.1	21.3	21.1	22.6

Table 63

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Individual Food Consumption (grams)

Group: I Control
Sex: Female

Animal	----Study Week----	
Number	1	2
1	66	75
2	72	81
3	70	70
4	75	84
5	77	72
6	80	88
7	69	101
8	114	107
9	65	72
10	84	92
11	90	90
12	69	78
13	90	62
14	88	95
15	82	100
16	97	97
17	51	102
18	88	96
19	62	68
20	75	81
Mean	78	86
gm/day	11.1	12.3

Table 64

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Individual Food Consumption (grams)

Group: II 10% OFC
Sex: Female

Animal Number	----Study Week---- 1	2
21	49	42
22	72	38
23	36	51
24	60	39
25	44	40
26	52	55
27	52	49
28	31	50
29	79	84
30	42	61
31	61	62
32	65	30
33	56	58
34	64	72
35	69	48
36	50	48
37	42	47
38	25	34
39	55	58
40	61	69
Mean	53	52
gm/day	7.6	7.4

Table 65

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Individual Food Consumption (grams)

Group: III 5% OFC
Sex: Female

Animal Number	----Study Week---- 1	2
41	104	92
42	85	89
43	74	98
44	102	91
45	88	96
46	101	91
47	77	79
48	89	99
49	106	110
50	104	103
51	102	93
52	74	96
53	85	92
54	70	78
55	105	100
56	84	75
57	110	114
58	91	75
59	115	120
60	125	118
Mean	95	95
gm/day	13.6	13.6

A summary of reproduction and lactation data noted for naturally cast litters is presented in Table 66. A discussion of these parameters follows. A summary of the indexes described is presented in Table 67.

Fertility

The percent of matings resulting in pregnancies was within normal limits for Control and 5% OFC groups. Some difficulty, however, was encountered with the 10% OFC animals wereby 22 males were necessary for the production of 13 litters from 20 females (total of naturally cast and caesarian derived) as compared to 20 males each needed to produce 17 Control litters and 16 5% OFC litters.

Gestation

One hundred percent of pregnancies in all groups resulted in litters cast alive. No alteration in duration of gestation was noted.

Viability

The viability index for 5% OFC animals (% of pups cast alive that survived to 4 days) was within normal limits and compared very favorably with control values (96.2% for 5% OFC and 89.8% for Control animals). A significant 4 day mortality in pups fed 10% OFC as well as an apparent reduction in litter size resulted in a depressed viability index (68.5%).

Table 66

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Summary of Reproduction and Lactation Indexes

	Group		
	I	II	III
Observations	Control	10% OFC	5% OFC
Number matings:	10	10	10
Mean wt., casting females, g:	330	292	309
Number surviving dams:	10	10	10
Number pregnancies:	9	7	8
Number males mated:	10	12	10
Gestation period, days:	22.2	22.3	22.1
Number litters cast alive:	9	7	8
Number pups cast alive:	79	51	84
cast dead:	0	4	0
alive at 4 days:	71	35	81
alive at 21 days:	57 of 59	16 of 29	57 of 58
Number pups per litter cast alive:	8.8	7.3	10.5
alive at 4 days:	7.9	5.0	10.1
alive at 21 days:	6.3	2.3	7.1
Mean body weight pups, g. cast alive:	6.9	4.7	7.1
alive at 21 days:	3.7	43.0	70.7
Abnormalities:	None	None	None
Indexes*			
Fertility	90	70	80
Gestation	100	100	100
Viability	89.8	68.5	96.2
Lactation	96.6	55.2	98.3

Fertility = percent of matings resulting in pregnancies

Gestation = percent of pregnancies resulting in litters cast alive

Viability = percent of pups cast alive that survived to 4 days

Lactation = percent of pups alive at 4 days that survived to weaning at 21 days (litters reduced to 8 pups at 4 days)

Lactation

Lactation performance for Control and animals fed 5% OFC were high whereas animals fed 10% again performed poorly, with a considerably higher mortality of pups during the 4 day to weaning period. Cannibalism of portions of two litters (26F and 36F) was noted in the 10% group which accounted for some of the mortality.

Table 67 contains the reproduction indexes noted.

Litter Growth

Litters from females fed 5% OFC were born heavier than Control animals and were larger at weaning. Ten percent OFC pups weighed significantly less at birth and remained smaller during the lactation period. Table 68 presents the average pup weights seen at birth and 21 days.

Tables 69-71 contain individual data for naturally cast litters including duration of gestation, pup numbers and survival, pup weights at birth and at weaning.

Table 67

REPRODUCTION INDEXES - PHASE I RATS, NATURALLY CAST LITTERS

	Group		
Index	I Control	II 10% OFC	III 5% OFC
Fertility	90	70	80
Gestation	100	100	100
Viability	89.8	68.5	96.2
Lactation	96.8	55.2	98.3

Table 68

AVERAGE BODY WEIGHTS OF PHASE I RAT PUPS AT BIRTH AND WEANING (21 days)

	Group		
Day	I Control	II 10% OFC	III 5% OFC
	(Average Body Weight (grams)		
0	6.9	4.7	7.1
21	63.7	43.0	70.7

Table 69

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Naturally Cast Litters: Individual Data Group: I Control

Mated Pairs

Animal Number		Dam Body Weight (gm)	Gestation Days	Pups Cast alive/dead	Pup Survival Day		Avg Pup Weights (gm) Day	
M	F				4	21	0	21
1	1	330	22	12/0				
3	3	317	22	15/0	11	8	6.5	68.3
5	5	310	23	8/0	8	8	7.4	66.0
6	6	328	22	13/0	12	8	6.0	65.7
8	8	316	21	3/0	3	3	8.5	70.3
10	10	337	22	5/0	4	3	8.0	69.3
12	12	370	23	4/0	4	4	5.3	60.1
13	13	259	N.P.[1]	- -	-	-	-	-
15	15	320	22	10/0	9	8	7.7	59.7
16	16	340	23	9/0	9	7	7.5	54.0
Mean[2]		330[3]	22.2	8.8/0	7.9	6.3	6.9	63.7

[1] Not pregnant

[2] 9 litters

[3] At casting, pregnant animals

Table 70

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Naturally Cast Litters: Individual Data Group: II 10% OFC

Mated Pairs

Animal Number M	F	Dam Body Weight (gm)	Gestation Days	Pups Cast alive/dead	Pup Survival Day 4	21	Avg Pup Weights (gm) Day 0	21
23	23	313	22	6/2	3	3	5.1	49.0
24	24	260	23	1/0	0	0	8.4	0
26	26	287	22	16/0	10	4	4.2	38.5
29	29	325	22	12/1	12	5	4.6	41.2
30	30	213	N.P.[1]	---	--	-	---	----
31	31	273	22	2/0	2	0	7.5	0
35	35	281	23	5/0	3	2	5.3	53.6
36	36	307	22	9/1	5	2	3.9	37.1
39,40	39	260	N.P.[1]	---	-	-	---	----
40,39	40	195	N.P.[1]	---	-	-	---	----
Mean[2]		292[3]	22.3	7.3/0.6	5.0	2.3	4.7	43.0

[1] Not pregnant

[2] 7 litters

[3] At casting, pregnant animals

Table 71

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

Naturally Cast Litters: Individual Data Group: III 5% OFC

Mated Pairs

Animal Number M	Animal Number F	Dam Body Weight (gm)	Gestation Days	Pups Cast alive/dead	Pup Survival Day 4	Pup Survival Day 21	Avg Pup Weights (gm) Day 0	Avg Pup Weights (gm) Day 21
43	43	290	21	12/0	12	8	6.1	74.5
44	44	361	21	18/0	18	8	6.0	73.1
48	48	325	23	14/0	12	8	6.2	64.2
51	51	225	N.P.[1]	--	--	-	---	----
52	52	280	23	7/0	7	7	8.9	70.0
53	53	240	N.P.[1]	--	--	-	---	----
54	54	300	23	12/0	11	8	7.4	68.3
55	55	315	23	10/0	10	7	8.5	72.8
58	58	301	22	3/0	3	3	9.1	84.6
59	59	298	21	8/0	8	8	8.3	67.2
Mean[2]		309[3]	22.1	10.5/0	10.3	7.1	7.1	70.7

[1] Not pregnant

[2] 8 litters

[3] At casting, pregnant animals

Incidence of Anomalies Among Litters Cast Naturally

There were no differences among groups, outside of body weight, which would be indicative of an untoward effect on fetal development of 5 or 10% levels of dietary OFC. Four stillborn pups were cast at the 10% OFC level as compared to none for the Control and 5% groups. This number of stillborn pups is, however, within the normal limit expected in a normal rat population.

Incidence of Anomalies in Females Sacrificed on the 13th Gestation Day

Table 72 summarizes the data from observations made during the 13-day parental female sacrifices.

Table 72

SUMMARY OF 13-DAY SACRIFICE OF PARENTAL FEMALES

Group	# Mated	# Pregnant	Corpora[1] Lutea left/right		Implant[1] Site left/right		Resorption[1] Site left/right	
I Control	10	8	4.3	4.0	4.0	3.8	0	0.1
II 10% OFC	10	6	2.7	1.7	2.9	1.5	0	0.1
III 5% OFC	10	8	4.1	4.3	3.8	4.5	0.2	0

[1] Average of 10 mated animals

A reduction in implant sites and corpora lutea were seen in 10% OFC animals. Corpora lutea, however, closely approximated the number of implant sites so that it was evident that there was no extensive resorption associated with the administration of the test material.

Control and 5% OFC animal ratios of implant sites to the number of corpora lutea were comparable as was the normal value obtained for total numbers of implant sites (and attached fetuses) seen in both groups.

Tables 73-75 contain individual data for the 13-day sacrificed parental females.

No skeletal or soft tissue abnormalities were seen in any pups delivered by section.

Table 73

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

13-Day Sacrifice of Parental Females: Individual Data
Group: I Control

Mated Pair Animal Number M	F	Body Weight (gms) Male Day 0	Female day 0	Female day 13	Corpora Lutea left	Corpora Lutea right	Implant Site left	Implant Site right	Resorption Site left	Resorption Site right
1	1	217	230	275						
2	2	330	238	256	2	5	0	0	0	0
3	3	320	207	248[1]						
4	4	272	178	216	7	4	7	4	0	0
5	5	278	190	224[1]						
6	6	224	222	252[1]						
7	7	339	207	237	3	5	5	5	0	0
8	8	280	217	245[1]						
9	9	321	239	270	7	1	3	6	0	0
10	10	325	226	262[1]						
11	11	306	266	299	5	5	5	5	0	0
12	12	298	280	313[1]						
13	13	301	223	252[1]						
14	14	315	206	226	0	0	0	0	0	0
15	15	241	222	257[1]						
16	16	240	218	237[1]						
17	17	237	196	229	4	5	5	4	0	0
18	18	272	195	222	6	6	6	6	0	0
19	19	310	271	296	5	3	5	3	0	0
20	20	255	231	270	4	6	4	5	0	1
Mean[2]					4.3	4.0	4.0	3.8	0	0.1

[1] Not Sacrificed

[2] Average of 10 sacrificed animals

Table 74

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

13-Day Sacrifice of Parental Females: Individual Data

Group: II 10% OFC

Mated Pair										
		Body Weight (gms)								
Animal Number		Male Day	Female day		Corpora Lutea		Implant Site		Resorption Site	
M	F	0	0	13	left/right		left/right		left/right	
21	21	292	155	167	0	0	0	0	0	0
22	22	208	203	225	5	4	7	3	0	0
23	23	240	230	251[1]						
24	24	240	178	200[1]						
25	25	224	222	243	3	1	3	0	0	1
26	26	185	216	232[1]						
27	27	287	193	224	4	4	4	5	0	0
28	28	215	232	250	7	0	7	0	0	0
29	29	260	241	260[1]						
30	30	260	231	247[1]						
31	31	294	207	228[1]						
32	32	257	160	179	3	7	3	7	0	0
33	33	260	207	217	0	0	0	0	0	0
34	34	228	205	213	0	0	0	0	0	0
35	35	180	211	235[1]						
36	36	174	207	240[1]						
37	37	220	227	240	5	0	5	0	0	0
38	38	252	163	179	0	0	0	0	0	0
39	40	231	178	188[1]						
40	39	220	161	175[1]						
Mean[2]					2.7	1.7	2.9	1.5	0	0.1

[1] Not Sacrificed

[2] Average of 10 Sacrificed Animals

Table 75

PHASE I - FERTILITY AND REPRODUCTION PERFORMANCE OF RATS FED OFC IN THE DIET

13-Day Sacrifice of Parental Females: Individual Data

Group: III 5% OFC

Mated Pair Animal Number M	F	Body Weight (gms) Male Day 0	Female day 0	13	Corpora Lutea left	right	Implant Site left	right	Resorption Site left	right
41	41	322	202	237	4	8	4	8	0	0
42	42	297	214	250	5	6	6	6	0	0
43	43	354	193	233[1]						
44	44	297	255	285[1]						
45	45	325	238	249	0	0	0	0	0	0
46	46	355	213	240	3	3	3	3	0	0
47	47	354	207	240	8	4	8	4	0	0
48	48	218	202	218[1]						
49	49	346	238	268	4	5	1	6	2	0
50	50	383	228	283	4	5	4	5	0	0
51	51	280	185	217[1]						
52	52	369	202	242[1]						
53	53	345	203	250[1]						
55	55	322	213	241[1]						
56	56	258	247	252	0	0	0	0	0	0
57	57	365	205	235	7	6	6	6	0	0
58	58	325	213	247[1]						
59	59	272	233	266[1]						
60	60	323	204	230	6	6	6	7	0	0
Mean[2]					4.1	4.3	3.8	4.5	0.2	0

[1] Not Sacrificed

[2] Average of 10 Sacrificed Animals

PHASE II - Teratology in Rats

The effects of 5 and 10% OFC administered in the diet, to rats, were evaluated by comparison of several parameters determined for both treatment and control groups. These parameters included:

- female survival and growth during gestation
- fetal viability and weight
- incidence of resorption sites
- tissue anomalies

Female Survival and Growth During Gestation

No deaths were recorded among animals of the control or test groups. Average body weight of parental females on OFC diets approximated or bettered that seen for control animals. Body weights seen at time of sacrifice are presented in Table 76.

Table 76

PARENTAL FEMALE BODY WEIGHT AT TIME OF SACRIFICE

Group	Body Wt. (grams)
I - Control	286
II - 10% OFC	295
III - 5% OFC	310

Fetal Viability and Weight

A summary of litter data are presented in Table 77.

Table 77

SUMMARY OF LITTER DATA, PHASE II - RATS

Group	Number of Litters	Number of Fetuses	Average Fetal Weight (gms)
I - Control	18	184	4.2
II - 10% OFC	18	225	3.9
III - 5% OFC	20	247	4.0

Two control fetuses failed to survive the 4-hour incubation period. No non-viable fetuses were seen at caesarean section of the dams. Thus, no treatment related effect on fetal viability was seen.

Fetal weights varied among diet groups with somewhat lower weights seen in the 10% OFC group. All weights were within the limits normally seen, however, and the effect, if any, of the 10% dietary OFC on fetal development was slight. Total litter weights and average fetal weights are presented in Tables 78-80.

Table 78

PHASE II - TERATOLOGY STUDY IN RATS FED OFC IN THE DIET

Summary of Litter Weights

Group: I Control

Dam Number	# of Fetuses	Total Litter Weight (gms)	Average Fetal Weight (gms)
1	9	38.7	4.3
2	4	21.6	5.4
3	6	36.6	6.1
4	11	37.4	3.4
5	2	6.8	3.4
6	0	0	0
7	13	42.8	3.3
8	14	53.2	3.8
9	12	72.0	6.0
10	13	56.0	4.3
11	12	42.0	3.5
12	10	43.0	4.3
13	10	62.5	6.3
14	12	42.0	3.5
15	6	30.0	5.0
16	13	44.2	3.4
17	11	39.6	3.6
18	16	60.8	3.8
19	0	0	0
20	12	42.0	3.5
Mean[1]	9.3	38.4	4.2

[1] 20 gestating females

Table 79

PHASE II - TERATOLOGY STUDY IN RATS FED OFC IN THE DIET

Summary of Litter Weights

Group: II 10% OFC

Dam Number	# of Fetuses	Total Litter Weight (gms)	Average Fetal Weight (gms)
21	14	67.2	4.8
22	10	62.0	6.2
23	17	52.7	3.7
24	13	37.7	2.7
25	14	43.4	3.1
26	13	42.9	3.3
27	11	33.0	3.3
28	0	0	0
29	0	0	0
30	12	30.0	2.5
31	10	31.0	3.1
32	12	44.4	3.7
33	13	44.4	3.4
34	14	39.2	2.8
35	15	63.5	4.2
36	15	66.2	4.4
37	14	49.8	3.6
38	13	42.8	3.3
39	9	44.0	4.9
40	12	42.0	3.5
Mean[1]	11.3	41.8	3.7

[1] 20 gestating females

Table 80

PHASE II - TERATOLOGY STUDY IN RATS FED OFC IN THE DIET

Summary of Litter Weights

Group: III 5% OFC

Dam Number	# of Fetuses	Total Litter Weight (gms)	Average Fetal Weight (gms)
41	14	44.5	3.2
42	14	43.2	3.1
43	11	38.6	3.5
44	10	39.7	4.0
45	13	40.3	3.1
46	9	36.7	4.1
47	10	36.8	3.7
48	13	49.5	3.8
49	14	65.1	4.7
50	11	36.5	3.3
51	11	44.4	4.4
52	13	57.3	4.4
53	12	53.1	4.4
54	11	47.5	4.3
55	13	63.5	4.9
56	13	68.7	5.2
57	13	69.9	5.4
58	15	71.9	4.8
59	14	80.4	5.7
60	13	39.0	3.0
Mean[1]	12.4	51.3	4.1

[1] 20 gestating females

Incidence of Resorption Sites

The absence of treatment effect on the incidence of resorption was reflected by the data presented in Table 81, which includes a summary of all reproductive parameters of gestating females. Tables 82-84 present the individual reproduciton parameters for parental females by diet group.

The total number of viable fetuses found at caesarean section closely approximated the number of implant sites. Thus, it was evident that there was no extensive resorption associated with the administration of the OFC diets.

Table 81

PHASE II - TERATOLOGY STUDY IN RATS FED OFC IN THE DIET

Summary of Reproduction Parameters of Gestating Females

	Group		
Observation	I Control	II 10% OFC	III 5% OFC
Number of gestating females	20	20	20
Total number Corpora Lutea	190	224	208
Total number implant sites	196	240	254
Total number viable fetuses	184	225	247
Total number non-viable fetuses[1]	2	0	0
Total number resorption sites	10	15	7
Number of dams with resorption sites	7	9	5
Average number Corpora Lutea	9.5	11.3	10.5
Average number implant sites	9.8	12.0	12.7
Average number viable fetuses	9.3	11.3	12.4
Average number non-viable fetuses	0.1	0	0
Average number resorption sites	0.5	0.8	0.4
Implant sites/Corpora Lutea (x100)	103.2	107.1	122.1
Fetuses/Corpora Lutea (x100)	96.8	100.4	118.8
Average weight of viable fetuses, g.	4.2	3.7	4.1
Average weight of dam[2], g.	286	295	310

[1] failed to survive incubation period
[2] at sacrifice

Table 82

PHASE II - TERATOLOGY STUDY IN RATS FED OFC IN THE DIET

Individual Reproduction Parameters for Parental Females

Group: I Control

Dam Number	Corpora Lutea		Implant Sites	Resorption Sites	Fetuses	4-hr. Incubation Survival
	R	L				
1	5	3	9	0	9	9
2	5	2	5	1	4	4
3	6	2	6	0	6	6
4	8	2	11	0	11	11
5	5	3	2	0	2	0
6	5	3	4	4	-	-
7	3	4	13	0	13	13
8	4	6	14	0	14	14
9	4	6	13	1	12	12
10	3	6	13	0	13	13
11	5	3	12	0	12	12
12	6	6	10	0	10	10
13	6	5	10	0	10	10
14	5	4	13	1	12	12
15	6	2	7	1	6	6
16	6	4	14	1	13	13
17	2	8	11	0	11	11
18	8	8	17	1	16	16
19	4	3	0	0	--	--
20	7	5	12	0	12	12
Mean[1]	5.2	4.3	9.8	0.5	9.3	9.2

[1] 20 animals

Table 83

PHASE II - TERATOLOGY STUDY IN RATS FED OFC IN THE DIET

Individual Reproduction Parameters for Parental Females

Group: II 10% OFC

Dam Number	Corpora Lutea R	Corpora Lutea L	Implant Sites	Resorption Sites	Fetuses	4-hr. Incubation Survival
21	7	6	14	0	14	14
22	4	7	10	0	10	10
23	8	4	17	0	17	17
24	6	4	14	1	13	13
25	7	4	14	0	14	14
26	8	4	13	1	13	13
27	6	4	11	0	11	11
28	7	6	0	0	--	--
29	3	6	5	5	--	--
30	6	8	12	0	12	12
31	7	3	11	1	10	10
32	6	4	12	0	12	12
33	8	2	15	2	13	13
34	7	6	14	0	14	14
35	6	5	17	2	15	15
36	5	7	16	1	15	15
37	8	8	14	0	14	14
38	7	2	14	1	13	13
39	3	5	10	1	9	9
40	4	6	12	0	12	12
Mean[1]	6.2	5.1	12.0	0.8	11.3	11.3

[1] 20 animals

Table 84

PHASE II - TERATOLOGY STUDY IN RATS FED OFC IN THE DIET

Individual Reproduction Parameters for Parental Females

Group: III 5% OFC

Dam Number	Corpora Lutea		Implant Sites	Resorption Sites	Fetuses	4-hr. Incubation Survival
	R	L				
41	8	3	14	0	14	14
42	6	5	15	1	14	14
43	2	8	11	0	11	11
44	8	2	11	1	10	10
45	7	5	15	2	13	13
46	6	2	10	1	9	9
47	2	8	10	0	10	10
48	2	6	13	0	13	13
49	2	8	14	0	14	14
50	8	3	13	2	11	11
51	4	5	11	0	11	11
52	6	5	13	0	13	13
53	8	3	12	0	12	12
54	5	6	11	0	11	11
55	2	7	13	0	13	13
56	2	8	13	0	13	13
57	7	2	13	0	13	13
58	8	6	15	0	15	15
59	3	8	14	0	14	14
60	7	5	13	0	13	13
Mean[1]	5.2	5.3	12.7	0.4	12.4	12.4

[1] 20 animals

Tissue Anomalies

Examinations of the soft tissues of pups taken by caesarean section showed no gross abnormalities. Incidental bone anomalies found on examination of the fetal skeletons are presented in Table 85.

Table 85

INCIDENCE OF BONE ANOMALIES, PHASE II - RATS

Bone Anomaly	Group	# of Litters	# of Fetuses
incomplete frontal ossification	I-Control	1	2
incomplete parietal ossification and/or separation of parietal bones	I-Control	3	17
	II-10%	2	10
	III-5%	2	15
scrambled ribs	I-Control	2	5
	II-10%	3	17
	III-5%	2	14
sternebrae not fully developed and/or bipartite	I-Control	4	19
	II-10%	5	40
	III-5%	2	15

None of the observed skeletal anomalies were considered remarkable. Most variations occurred within all pups in a litter and were not considered treatment related. Summaries of individual skeletal examinations are presented in Tables 86-88

Table 86

PHASE II - TERATOLOGY STUDY IN RATS FED OFC IN THE DIET

Summary of Skeletal Observations for Individual Parental Females

Group: I Control

Dam Number	Number of Pups Examined		Skeletal Observations
1	6		none
2	2	2 pups:	incomplete frontal ossification
		2 pups:	scrambled ribs
		2 pups:	sternebrae not fully developed
3	4	1 pup:	separation of parietal bones
4	7		none
5	1	1 pup:	sternebrae not fully developed and bipartite
6	0		-
7	8		none
8	10		none
9	8		none
10	8		none
11	8	8 pups:	incomplete ossification and separation of parietal bones
		8 pups:	sternebrae not fully developed and bipartite
12	6		none
13	6		none
14	8		none
15	4		none
16	8	8 pups:	incomplete ossification and separation of parietal bones
		8 pups:	sternebrae not fully developed and bipartite
17	7	1 pup:	12 pair of ribs
18	8		none
19	0		-
20	8	3 pups:	scrambled ribs

Table 87

PHASE II - TERATOLOGY STUDY IN RATS FED OFC IN THE DIET

Summary of Skeletal Observations for Individual Parental Females

Group: II 10% OFC

Dam Number	Number of Pups Examined		Skeletal Observations
21	10		none
22	6		none
23	12		none
24	8	8 pups:	incomplete ossification and separation of parietal bones
		8 pups:	sternebrae not fully developed and bipartite
25	8		none
26	8		none
27	8	1 pup:	scrambled ribs
		8 pups:	sternebrae not fully developed
28	0		-
29	0		-
30	8	8 pups:	sternebrae not fully developed
31	6	6 pups:	sternebrae not fully developed and bipartite
		6 pups:	scrambled ribs
32	8		none
33	8		none
34	10	10 pups:	scrambled ribs
		10 pups:	sternebrae not fully developed and bipartite
		10 pups:	incomplete ossification and separation of parietal bones
35	10		none
36	10		none
37	8		none
38	8		none
39	6		none
40	8		none

Table 88

PHASE II - TERATOLOGY STUDY IN RATS FED OFC IN THE DIET

Summary of Skeletal Observations for Individual Parental Females

Group: III 5% OFC

Dam Number	Number of Pups Examined	Skeletal Observations
41	10	none
42	10	none
43	8	none
44	6	none
45	8	none
46	6	none
47	6	6 pups: scrambled ribs
48	8	none
49	10	none
50	7	7 pups: incomplete ossification and separation of parietal bones 7 pups: sternebrae not fully developed
51	7	none
52	8	none
53	8	none
54	8	none
55	8	none
56	8	none
57	8	none
58	10	none
59	8	none
60	8	8 pups: incomplete ossification and separation of parietal bones 8 pups: sternebrae not fully developed and bipartite 8 pups: scrambled ribs

Phase II - Teratology in Rabbits

The effects of 5 and 10% OFC administered in the diet, to rabbits, were evaluated by comparison of several parameters determined for both treatment and control groups. These parameters included:

- female survival and growth during gestation
- fetal viability and weight
- incidence of resorption sites
- 18-hour viability of incubated pups
- soft-tissue and skeletal observations

Female Survival and Growth During Gestation

All animals survived the study period. The average body weight of parental females at term were similar for Control and 5% OFC animals but were somewhat reduced at the 10% OFC level. Body weights seen at the time of sacrifice are presented in Table 89.

Table 89

BODY WEIGHT OF DOES AT TIME OF SACRIFICE

Group	Body Weight (kg)
I - Control	3.8
II - 10% OFC	3.3
III - 5% OFC	4.0

Fetal Viability and Weight

A summary of litter data is presented in Table 90.

Table 90

SUMMARY OF VIABLE LITTER DATA, PHASE II - RABBITS

Group	Number of Litters	Number of Fetuses	Average Fetal Weight (grams)
I - Control	8	59	47.5
II - 10% OFC	7	66	42.8
III - 5% OFC	7	48	48.0

One 10% OFC pup and one 5% OFC pup failed to survive the 18-hour incubation period. Two Control litters totaling 8 fetuses were found non-viable at caesarean section and were partially resorbed. These occurrences were not deemed treatment related.

Fetal weights varied among diet groups. Slightly lower average fetal weights were seen in the larger number of 10% OFC pups than in the Control and 5% OFC animals. Average fetal weights are presented in Tables 91-93.

Table 91

PHASE II - TERATOLOGY STUDY IN RABBITS FED OFC IN THE DIET

Summary of Fetal Weights

Group: I Control

Doe Number	# of Fetuses	Average Fetal Weight (gms)
1	7	50.7
2	9	62.5
3	6	64.0
4	5	37.5
5	7	48.2
6	10	35.0
7	6	65.2
8	9	26.0
9	0	-
10	0	-

Table 92

PHASE II - TERATOLOGY STUDY IN RABBITS FED OFC IN THE DIET

Summary of Fetal Weights

Group: II 10% OFC

Doe Number	# of Fetuses	Average Fetal Weight (gms)
11	9	41.0
12	-	-
13	9	68.1
14	7	30.0
15	11	40.1
16	-	-
17	7	42.1
18	11	39.7
19	12	38.4
20	-	-

Table 93

PHASE II - TERATOLOGY STUDY IN RABBITS FED OFC IN THE DIET

Summary of Fetal Weights

Group: III 5% OFC

Doe Number	# of Fetuses	Average Fetal Weight (gms)
21	10	47.9
22	3	61.0
23	2	64.3
24	9	39.2
25	0	-
26	4	57.5
27	-	-
28	10	41.3
29	-	-
30	10	51.6

Incidence of Resorption Sites

The majority of resorption sites were found in two litters of the control group. The absence of treatment effect on the incidence of resorption was reflected by the data present in Table 94 which also includes a summary of all reproductive parameters studied. Tables 95-97 present the individual reproduction parameters for parental females by diet group.

The total number of viable fetuses found at caesarean section closely approximated the number of implant sites for treated animals. Thus, it was evident that there was no extensive resorption associated with the administration of the OFC diets.

Table 94

PHASE II - TERATOLOGY STUDY IN RABBITS FED OFC IN THE DIET

Summary of Reproduction Parameters of Gestating Females

		Group	
Observation	I Control	II 10% OFC	III 5% OFC
Number of gestating females	10	7	8
Total Number Corpora Lutea	83	60	59
Total number implant sites	70	67	53
Total number viable fetuses	59	66	48
Total number non-viable fetuses[1]	0	1	1
Total number resorption sites	11	1	5
Number of does with resorption sites	5	1	3
Number of does with viable litters	8	7	7
Average number Corpora Lutea	8.3	8.6	7.4
Average number of implant sites	7.0	9.6	6.6
Average number of viable fetuses	5.9	9.4	6.0
Average number non-viable fetuses	0	0.1	0.1
Average number resorption sites	1.1	0.1	0.6
Implant sites/Corpora Lutea (x100)	84.3	111.7	89.8
Fetuses/Corpora Lutea (x100)	71.1	110.0	81.4
Average weight of viable fetuses (g)	47.5	42.8	48.0
Average weight of doe[2] (kg)	3.8	3.3	4.0

[1] failed to survive incubation period

[2] at sacrifice

Table 95

PHASE II - TERATOLOGY STUDY IN RABBITS FED OFC IN THE DIET

Individual Reproduction Parameters for Parental Females

Group: I Control

Doe Number	Corpora Lutea		Implant Sites	Resorption Sites	Fetuses	18-hr. Incubation Survival
	R	L				
1	5	3	8	1	7	7
2	4	3	9	0	9	9
3	4	5	7	1	6	6
4	8	5	5	0	5	5
5	5	3	7	0	7	7
6	6	2	11	1	10	10
7	2	6	6	0	6	6
8	3	3	9	0	9	9
9	4	4	5	5	0	0
10	3	5	3	3	0	0
Mean[1]	4.4	3.9	7.0	1.1	5.9	5.9

[1] 10 pregnant animals

Table 96

PHASE II - TERATOLOGY STUDY IN RABBITS FED OFC IN THE DIET

Individual Reproduction Parameters for Parental Females

Group: II 10% OFC

Doe Number	Corpora Lutea		Implant Sites	Resorption Sites	Fetuses	18-hr. Incubation Survival
	R	L				
11	4	3	9	0	9	9
12	N.P.[1]		-	-	-	-
13	2	4	9	0	9	9
14	4	4	7	0	7	7
15	7	2	12	1	11	10
16	N.P.[1]		-	-	-	-
17	3	3	7	0	7	7
18	5	4	11	0	11	11
19	5	5	12	0	12	12
20	N.P.[1]		-	-	-	-
Mean[2]	4.3	4.3	9.6	0.1	9.4	9.3

[1] not pregnant

[2] 7 pregnant animals

Table 97

PHASE II - TERATOLOGY STUDY IN RABBITS FED OFC IN THE DIET

Individual Reproduction Parameters for Parental Females

Group: III 5% OFC

Doe Number	Corpora Lutea		Implant Sites	Resorption Sites	Fetuses	18-hr. Incubation Survival
	R	L				
21	4	2	10	0	10	10
22	3	6	4	1	3	3
23	3	5	3	1	2	1
24	3	2	9	0	9	9
25	3	4	3	3	0	0
26	3	4	4	0	4	4
27	N.P.[1]		-	-	-	-
28	4	3	10	0	10	10
29	N.P.[1]		-	-	-	-
30	4	6	10	0	10	10
Mean[2]	3.4	4.0	6.6	0.6	6.0	5.9

[1] not pregnant

[2] 8 pregnant animals

Litter Size

The excess of fetuses over corpora lutea in the 10% OFC group, and the larger litter size than that seen in Control and 5% OFC animals may be indicative of an increase in multiple births within some litters of the 10% OFC group. Table 98 presents this data.

Table 98

INCREASED INCIDENCE OF MULTIPLE BIRTHS: PHASE II - RABBITS

	Group		
	I - Control	II - 10%	III - 5%
Implant sites/corpora lutea (x100)	84.3	111.7	89.8
Fetuses/corpora lutea (x100)	71.1	110.0	81.4
Total fetuses	59	66	48
Total viable litters	8	7	7
# of litters with excess of fetuses over corpora lutea	3	6	3

However, since the period of treatment was subsequent to ovulation, fertilization and nidation, the multiple birth increase cannot be treatment related and must be an artifact of normal rabbit reproduction.

Soft Tissue and Skeletal Observations

No remarkable anomlies were seen on either soft-tissue or skeletal examination except for one ventricular septal defect seen in a non-viable pup in the 5% OFC group. This defect was not considered treatment related. Skeletal observations included fetuses with supernumary ribs and underdeveloped or an occasional absent sternebrae. These observations were considered to be a function of the time from conception to section rather than a treatment- or does-related effect since they were seen in Control does as well as in does fed 5 and 10% levels of OFC diets.

In the absence of any significant findings, it was concluded that the feeding of 5 and 10% OFC in the diet did not alter the development of normal bone or soft-tissue structures. The OFC diets were therefore not considered teratogenic.

Incidental bone anomalies found on examination of the fetal skeletons are presented in Table 99. Summaries of individual skeletal examinations are presented in Tables 100-102.

Table 99

INCIDENCE OF BONE ANOMALIES, PHASE III RABBITS

Bone Anomaly	Group	# of Litters	# of Fetuses
# 5 sternebrae underdeveloped or absent	I - Control	4	16
	II - 10%	3	24
	III - 5%	3	16
#6 sternebrae underdeveloped or absent	I - Control	3	13
	II - 10%	3	24
	III - 5%	2	9
Other sternebrae underdeveloped	I - Control	1	3
	II - 10%	0	0
	III - 5%	0	0
Extra rib (bilateral)	I - Control	2	3
	II - 10%	1	3
	III - 5%	0	0
Extra rib (unilateral)	I - Control	1	1
	II - 10%	1	1
	III - 5%	0	0

Other Anomalies	
Poorly ossified parietal bones:	1 pup, 10% OFC
Neck twist:	2 pups, 1 litter, 5% OFC
Partially resorbed pups:	8 pups, 2 litters, Control 3 pups, 1 litter, 5% OFC
Ventricular septal defect:	1 non-viable pup, 5% OFC

Table 100

PHASE II - TERATOLOGY STUDY IN RABBITS FED OFC IN THE DIET

Summary of Skeletal Observations for Individual Parental Females

Group: I Control

Doe Number	Number of Pups Examined	Skeletal Observations	
1	4	4 pups:	#5 and #6 Sternebrae underdeveloped
		1 pup:	extra rib (bilateral)
2	6	3 pups:	#5 sternebrae underdeveloped
		3 pups:	#5 sternebrae absent
		6 pups:	#6 sternebrae underdeveloped
3	4		none
4	3	3 pups:	#1, #2, #3, #4 and #6 sternebrae underdeveloped
		3 pups:	#5 sternebrae absent
5	4		none
6	6	6 pups:	#5 sternebrae underdeveloped
		2 pups:	extra rib (bilateral)
7	4	1 pup:	extra rib (unilateral)
8	6		none
9	0		5 pups partially resorbed
10	0		3 pups partially resorbed

Table 101

PHASE II - TERATOLOGY STUDY IN RABBITS FED OFC IN THE DIET

Summary of Skeletal Observations for Individual Parental Females

Group: II 10% OFC

Doe Number	Number of Pups Examined	Skeletal Observations	
11	6		none
12	-		none
13	6		none
14	6		none
15	8	8 pups:	#5 sternebrae underdeveloped
		8 pups:	#6 sternebrae absent
		3 pups:	extra rib (bilateral)
		1 pup:	poorly ossified parietal bones causing concave cranium
		1 pup:	non-survivor of incubator: no anomalies
16	-		-
17	4	1 pup:	extra rib (unilateral)
		3 pups:	mechanically damaged ribs
18	8	8 pups:	#5 and #6 sternebrae underdeveloped
19	8	3 pups:	#5 sternebrae absent
		3 pups:	#6 sternebrae underdeveloped
		5 pups:	#5 sternebrae underdeveloped
		5 pups:	#6 sternebrae absent
20	-		-

Table 102

PHASE II - TERATOLOGY STUDY IN RABBITS FED OFC IN THE DIET

Summary of Skeletal Observations for Individual Parental Females

Group: III 5% OFC

Doe Number	Number of Pups Examined	Skeletal Observations	
21	7		none
22	2	2 pups:	#5 sternabrae underdeveloped
		2 pups:	#6 sternebrae absent
		2 pups:	neck twisted to left
23	1		none
		1 pup:	non-survivor of incubator found with ventricular septal defect
24	6		none
25	0		3 pups partially resorbed
26	2		none
27	-		-
28	7	7 pups:	#5 sternebrae underdeveloped
29	-		-
30	7	7 pups:	#5 sternebrae absent
		7 pups:	#6 sternebrae absent

Phase III - Perinatal and Postnatal Study in Rats

The effects of dietary administration of OFC at levels of 5 and 10% were evaluated by comparison of several parameters determined for treatment and control groups. These parameters included:

- body weights of treated dams
- number of young, live and dead, cast at parturition
- viability of young during lactation
- growth of young during lactation

Parental Survival and Growth

One Control female (Dam #7) died following the fourth postgestational day, resulting in the death of all her offspring. No reproductive cause of death was ascertained. Gross necropsy revealed significant pneumonic lesions to which her death was attributed. All other parental females survived the study period.

Although body weights of 10% OFC female parents were slightly lower than Control and 5% OFC animals at term, all parents maintained a similar growth pattern during lactation. During the lactation period, pronounced changes in body weights are not usually seen. Body weight data are presented in Table 103.

Table 103

AVERAGE BODY WEIGHTS - PHASE III PARENTAL FEMALE RATS

	Body Weight in Grams			
Group	Before Casting	After Casting	Day 1 Post Casting	Day 21 Weaning
I - Control	344	268	273	277
II - 10%	313	248	256	247
III - 5%	343	259	252	255

Individual Body Weight Data are summarized in Table 104-106.

Table 104

PHASE III - PERINATAL AND POSTNATAL STUDY IN RATS FED OFC IN THE DIET

Naturally Cast Litters: Individual Data

Group: I Control

	Dam Body Weight (gms)				Pups				
						Survival Day		Weight (gm) Day	
Dam No.	Before Casting	After Casting	Day 1 Post Casting	Day 21 Weaning	Cast Alive/Dead	4	21	0	21
1	352	250	255	270	8/0	8	8	8.8	60.4
2	377	272	280	305	14/0	13	8	7.4	60.8
3	361	270	272	270	13/0	13	7	7.1	62.1
4	303	260	264	270	8/0	8	8	7.3	37.0
5	360	285	280	290	13/0	11	8	7.0	54.5
6	348	268	278	268	13/0	11	8	7.4	70.2
7	282	277	270	-Died-	13/0	7	0	7.7	0
8	337	260	284	300	11/0	11	8	8.2	60.0
9	315	280	275	287	6/0	6	5	5.9	41.5
10	377	280	295	305	15/0	15	6	6.8	75.3
11	375	295	304	300	11/0	11	5	8.1	50.9
12	340	255	250	247	11/0	11	8	7.2	78.6
13	N.P.[1]								
14	315	250	262	238	11/0	11	8	7.0	56.4
15	351	268	265	287	14/0	13	8	6.6	61.3
16	379	290	286	275	13/0	13	6	6.8	77.8
17	292	225	231	243	11/0	11	8	7.3	79.2
18	345	250	275	257	13/0	12	7	6.2	57.2
19	378	280	295	300	14/0	14	5	7.7	60.2
20	N.P.[1]								
Mean:	344	268	273	277	11.8/0	11.1	7.1	7.3	61.4

[1] not pregnant

Table 105

PHASE III - PERINATAL AND POSTNATAL STUDY IN RATS FED OFC IN THE DIET

Naturally Cast Litters: Individual Data

Group: II 10% OFC

	Dam Body Weight (gms)				Pups				
						Survival Day		Weight (gm) Day	
Dam No.	Before Casting	After Casting	Day 1 Post Casting	Day 21 Weaning	Cast Alive/Dead	4	21	0	21
21	310	263	288	265	2/0	0	0	8.0	0
22	295	241	251	250	7/1	4	2	6.2	39.7
23	293	224	219	207	14/1	6	2	7.1	13.1
24	300	240	257	253	6/4	6	4	4.7	42.5
25	287	212	234	238	9/0	9	8	7.9	63.0
26	314	252	257	241	4/0	4	1	5.4	49.2
27	929	195	202	191	5/0	2	0	4 5	0
28	334	270	267	275	8/3	7	4	[illegible] 1	40.0
29	316	240	253	253	0/10	0	0	0	0
30	296	254	252	247	8/0	8	5	5.2	54.0
31	358	282	271	239	9/0	3	0	4.9	0
32	256	235	242	248	0/3	0	0	0	0
33	378	295	314	299	8/0	6	0	3.7	0
34	343	255	253	212	2/0	0	0	6.4	0
35	292	223	246	227	17/0	0	0	5.6	0
36	N.P.[1]								
37	363	280	277	279	8/0	8	8	6.7	52.7
38	297	250	262	268	4/0	4	3	7.4	64.2
39	N.P.[1]								
40	N.P.[1]								
Mean:	313	248	256	247	7.4/1.5	3.7	2.5	5.9	50.6

[1] not pregnant

Table 106

PHASE III - PERINATAL AND POSTNATAL STUDY IN RATS FED OFC IN THE DIET

Naturally Cast Litters: Individual Data

Group: III 5% OFC

	Dam Body Weight (gms)				Pups				
						Survival Day		Weight (gm) Day	
Dam No.	Before Casting	After Casting	Day 1 Post Casting	Day 21 Weaning	Cast Alive/Dead	4	21	0	21
41	315	267	258	250	9/0	9	6	6.3	55.2
42	370	267	253	270	14/0	13	7	5.9	60.5
43	318	252	248	247	11/0	11	8	7.1	61.5
44	337	258	258	258	13/0	13	4	7.0	67.6
45	330	220	243	247	13/0	12	7	6.7	76.9
46	338	250	247	257	14/0	10	6	6.4	50.0
47	320	242	244	248	13/0	13	8	5.2	61.8
48	N.P.[1]								
49	373	288	280	267	12/0	12	6	5.8	57.5
50	350	263	250	247	14/0	14	8	5.3	70.0
51	344	272	260	258	11/0	11	8	7.7	77.7
52	345	274	255	266	11/0	11	5	6.7	45.1
53	362	290	273	278	14/0	14	4	5.7	65.0
54	330	250	243	252	11/0	11	5	6.8	62.3
55	337	248	242	242	9/0	9	8	7.0	48.6
56	362	252	250	252	16/0	16	5	6.4	48.8
57	380	283	270	283	12/0	12	8	6.5	78.9
58	338	260	248	254	13/0	12	7	5.5	73.4
59	343	240	240	228	14/0	12	7	6.2	70.7
60	320	240	233	241	13/0	13	3	7.3	71.3
Mean:	343	259	252	255	12.5/0	12.0	6.3	6.4	63.3

[1] not pregnant

Number of Young Cast at Parturition

Tables 104 to 106 present, in addition to body weight data, all individual litter size and growth information derived during this study.

The total number of pups cast alive and the mean number of pups per litter were comparable among the Control and 5% OFC groups. A significant decrease in live births and pups per litter was recorded for the 10% OFC level. Table 107 presents the total and average individual litter size data.

Table 107

CASTING OF PUPS - PHASE III RATS

Group	Total Number of Pups Cast Alive	Total Number of Pups Cast Dead	Average Litter Size
I - Control	212	0	11.8
II - 10%	111	22	7.4
III - 5%	237	0	12.5

Disparity was also noted in numbers of litters cast alive among the Control and 5% and the 10% OFC groups. Table 108 presents these data.

Table 108

LITTERS CAST ALIVE - PHASE III RATS

Group	Number of Pregnancies	Number of Litters Cast Alive	Number of Litters Cast Dead
I - Control	18	18	0
II - 10%	17	15	2
III - 5%	19	19	0

Table 109 relates the litter sizes with the total number of litters produced.

Table 109

DISTRIBUTION OF LITTER SIZES - PHASE III RATS

Litter Size	Group I - Control	II - 10% OFC	III - 5% OFC
0	2	5	1
1-5	0	5	0
6-10	3	8	2
11-15	15	1	16
16-20	0	1	1
TOTAL	20	20	20

Viability of Young During Lactation

In this controlled study in which the survivial indexes were compared for both control and treated groups, there were no obvious effects on the 5% OFC young as compared with the controls during the nursing period. The 10% OFC viability index was greatly reduced due to a large neonatal mortality from 0 to 4 post-gestational days. Mortality for this group continued at a lesser degree through the 21-day lactation period. Table 110 presents these data.

Table 110

VIABILITY OF PUPS - PHASE III RATS

Group	Mortaltiy Day 0 - 4	Day 4 - 21
I - Control	13 of 212	20 of 141
II - 10% OFC	56 of 111	17 of 54
III - 5% OFC	9 of 237	32 of 152

One control litter of 7 was sacrificed after the 4-day observation due to the death of the dam. Three 10% OFC litters were canabalized as were 5 of 8 pups in one 5% OFC group.

Viability and Lactation Indexes as well as summary of all reproductive parameters for Phase III rats are presented in Table 111.

Table 111

PHASE III - PERINATAL AND POSTNATAL STUDY IN RATS FED OFC IN THE DIET

Summary of Reproduction and Lactation Indexes

	Groups		
	I	II	III
Observations	Conrol	10% OFC	5% OFC
Number matings:	20	20	20
Mean wt; casting females, g:	268	248	259
Mean wt; dams at weaning, g:	277	247	255
Number surviving dams:	19	20	20
Number pregnancies:	18	17	19
Number litters cast alive:	18	15	19
Number pups cast alive:	212	111	237
Number pups cast dead:	0	22	0
Number pups alive at 4 days:	199	55	228
alive at 21 days:	121 of 141	37 of 54	120 of 152
Number pups per litter cast alive:	11.8	7.4	12.5
Number pups alive at 4 days:	11.1	3.7	12.0
alive at 21 days:	7.1	2.5	6.3
Mean body weight pups, g, cast alive:	7.3	5.9	6.4
alive at 21 days:	61.4	50.6	63.3
Abnormalities:	None	None	None
Indexes*			
Fertility	90	85	95
Gestation	100	88.2	100
Viability	93.9	49.5	96.2
Lactation	85.8	68.5	78.9

Growth of Surviving Young During Lactation

Table 112 presents the average body weights of pups during the lactation period.

Table 112

PUP GROWTH DURING LACTATION - PHASE III RATS

	Body Weight (grams)	
Group	At Casting	At Weaning
I - Control	7.3	61.4
II - 10%	5.9	50.6
III - 5%	6.4	63.3

A lessened growth rate was evident for 10% OFC rats during this period when compared with control and 5% OFC animals.

Necropsy Findings

No soft-tissue abnormalities were found at necropsy of the weanlings. Similarly, no abnormalities were seen in those pups sacrificed at the fourth post-partum day during the culling process.

Egg Production in Chickens

The egg-laying performance of chickens fed the synthetic diet containing varying concentrations of OFC is presented in Table 113.

Table 113

OFC SYNTHETIC DIETS

Egg-laying Performance: Average Daily Egg Production For Each Experimental Group (9 birds)

	Daily Egg Production						
	Week						
Group	0	1	2	3	4	5	6
I Control	6.1	5.6	6.0	6.0	6.1	5.7	6.2
II 5% OFC	5.7	5.7	4.1	4.0	4.2	4.5	4.1
III 10% OFC	6.5	4.3	2.0	1.1	0.8	0.5	0.5
IV 15% OFC	6.8	3.4	1.1	0	0	0	0
V 20% OFC	6.3	2.1	0	0	0	0	0

Decreased egg production for animals in Groups III, IV and V were apparent at the end of the initial week of diet.

The effect on egg production by animals in Group II was seen at the conclusion of the second week. In contrast to the pattern of decreasing egg production as a function of time noted in chickens of Groups III, IV and V, continued feeding of the 5% OFC diet to animals in Group II did not result in a suppression of egg-laying performance beyond that seen at the conclusion of the second week of feeding.

In the two animals from each group sacrificed at the conclusion of this phase of the study, no gross lesions were seen at necropsy.

The effect on egg production of a dietary change to commercial chow is presented in Table 114.

Table 114

EGG-LAYING PERFORMANCE FOLLOWING RETURN TO CONTROL (PURINA) DIET: AVERAGE DAILY EGG PRODUCTION (PER 7 BIRDS)

	Daily Egg Production			
	Week			
Group	1	2	3	4
I	5.8	6.5	6.8	6.5
II	5.0	5.5	6.0	6.1
III	4.8	6.0	6.4	6.5
IV	2.2	5.8	6.5	6.2
V	1.1	4.8	5.5	5.2

The return of animals to the poultry diet was followed by a return to normal egg production by chickens in Groups II, III and IV. Although egg production by chickens in Group V was increased with the return to the control diet, it remained somewhat lower than that for birds of the other groups.

The results obtained when OFC levels were added to the commercial chicken feed are presented in Table 115.

Table 115

EGG-LAYING PERFORMANCE FOLLOWING FEEDING OF SLUDGE-ADDED COMMERCIAL DIET: AVERAGE DAILY PRODUCTION (PER 7 BIRDS)

Group	Week 1	Week 2	Week 3	Week 4
I Control (Purina)	6.5	6.0	6.1	5.9
II Purina-5% OFC	6.1	5.2	4.1	3.5
III Purina-10% OFC	4.8	2.2	0.5	1.0
IV Purina-15% OFC	2.8	1.0	0	0
V Purina-20% OFC	1.7	0	0	0

It was evident in all egg production studies, that all levels of OFC diets, synthetic or commercial, produced a severe decrease in egg production proportional to the amount of OFC in the diet. This process was reversible, however, as subsequent feeding of non-OFC diets indicated.

The Effect on Taste of Eggs Obtained From OFC Fed Chickens

The eggs used in the test on June 19, 1972, were comparable and showed normal variation in yolk color. No other differences were observed. The shells of the OFC eggs used June 29, 1972, however, were very fragile and broke too easily, and were not as hard as those of the control eggs. Normal variation in yolk color was observed between and among the eggs.

For the eggs layed June 29, 1972 those from Control chickens were a lighter yellow than those from OFC fed chickens.

In order to obviate any differences in yolk colors, lights were dimmed in the panel room where the triangle tests were conducted.

The results of the triangle tests are summarized in Table 116. No statistically significant differences were found between the two samples of June 19, 1972.

Statistically highly significant differences were found between the samples tested June 29, 1972. Where the OFC sample was the odd sample it was described as being not as "eggy" and as having more flavor or, being metallic and a non-fresh taste. Where the control was the odd sample; it was described as tasting better, fresher, having a real egg flavor, as having less flavor, and being less metallic.

Eggs obtained August 23, 1972 from chickens fed 5% OFC could be distinguished from control eggs at a 5% significance level.

Table 116

SUMMARY OF TRIANGLE TESTS - CONTROL EGGS VS EGGS FROM CHICKENS FED OFC

Date	Total Observations	Correct Judgments	Statistical Significance
6/19/72	20	7	Insignificant
6/29/72	14	12	0.1%
8/23/62	18	10	5.0%

General Considerations

Earlier studies had shown that Organic Feed Concentrate obtained by SO_2 hydrolysis of activated sludge provides energy equivalent to that of sugar cane molasses when pair-fed to rats at the 10% level in the diet.

In the present study, the effect of OFC on several toxicological parameters was measured. These were as follows:

- Fertility and reproduction in rats
- Teratologic potential in rats and rabbits
- Parturition and neonatal development in rats
- Egg-laying production in chickens

The data clearly show that no teratologic abnormalities occurred nor was there an effect on the gestation period when OFC was fed at either the 5% or 10% level in the diet. Normal results were obtained for the following:

- Total number of corpora lutea
- Total number of implant sites

- Total number of viable fetuses
- Total number on non-viable fetuses
- Total number of resorption sites
- Bone anamolies in the fetus of rats and rabbits
- Soft tissue structure

Further, at the 5% level in the diet, OFC produced no observable effect on fertility, reproduction, parturition and neonatal development in the rat. Egg-laying was slightly reduced at the 5% level.

However, at the 10% level in the diet several parameters were unsatisfactorily influenced.

- Adult rats gained less weight and ate less
- Decreased fertility in rats
- Decreased 4 day mortality (viability) in rat pups
- Reduction in litter size
- Increased mortality of pups during weaning
- Decreased weight of pups at birth and reduced rate of growth
- Increased number of pups cast dead
- Decreased litter size
- Decreased egg-laying in chickens

An attempt was made to interpret these data in the light of other obtainable facts.

A mineral analysis of the OFC used in the present study is presented in Table 117. The ash content of 14.6% is comparable to that normally found in sugar cane molasses. However, heavy metals including iron, magnesium, copper, nickel and lead are relatively high. Further, the fluorine level was 650 ppm, which at the 10% level of OFC in the diet represents 65 ppm of fluorine, or more than 10 times the normal level of this halogen

Table 117

SPRAY DRIED ORGANIC FEED CONCENTRATE (14.6% ASH)

Emission Spectro on ash - semi-quantitative

%	Element
10-100	P
1-10	Fe, Mg, Na, Ca
0.1-10	Si
.01-0.1	Al, Mn, Cu, Cr, Ti, Ni, B, Ba, Pb
.001-.01	Sn, Ag

Corrected to original spray dried solids

%	ppm	Element
1.5-15	15000 - 150000	P
.15-1.5	1500 - 15000	Fe, Mg, Na, Ca
.015-.15	150 - 1500	Si
.0015-.015	15 - 150	Al, Mn, Cu, Cr, Ti, Ni, B, Ba, Pb
.00015-.0015	1.5 - 15	Sn, Ag

Cobalt none detected
Fluorine .065% 650 ppm

Previous samples of OFC were also found to be high in iron, but essentially normal in levels of sodium, potassium and magnesium. In particular, these earlier specimens were low in phosphorus. Thus, depending on the source of the activated sludge, the OFC may contain variable quantities of heavy metals and phosphorus, which can markedly affect the acceptability of the OFC.

The high phosphorus content of the OFC used in the present study also can account for the great reduction of egg-laying by chickens fed the 10% OFC diet. The relatively (although not absolute) low level of calcium influences shell formation in the eggs. In this respect, the egg shells, even in those eggs layed, were softer than normal. It is interesting that the OFC was found to possess strong calcium binding properties.

Thus, treatment of the activated sludge to remove a major part of the heavy metals and phosphorus, before produciton of OFC, should eliminate the unsatisfactory properties noted at the 10% level in the diet.

At the 5% level of OFC in the diet, not only was there no toxicity observed, but there appeared to be growth stimulating properties in the preparation.

An amino acid analysis of the OFC used in this study is presented in Table 118. This analysis was performed by column chromatography according to the procedure of Stein and Moore. The data indicate that OFC contains approximately 22% protein. Similar to other proteins of bacterial origin it is low in the sulfur amino acids methionine and cystine (Table 119). Compared to egg protein - taken as a standard - OFC contains only 38% as much sulfur amino acids. However, other essential dietary amino acids are present in reasonably sufficient amounts so that the total estimated biological value is almost 78%, which is equivalent to meat protein and higher than that of casein. For ruminants such as cattle and sheep, this is not important, since any dietary nitrogen (or protein) is converted in the rumen to the normal bacterial protein; but for pigs and chicken this could represent a plus dietary factor. For chickens, the reasonably high levels of glycine and arginine in OFC are important considerations. Comparing OFC to sugar cane molasses, it would not be necessary to add urea to cattle feed when using OFC as a feed constituent.

Table 118

AMINO ACID ANALYSIS OF OFC USED IN TERATOLOGY STUDY

Amino Acid	Percent
Lysine	1.58
Histidine	0.49
Arginine	0.86
Aspartic Acid	1.77
Threonine	1.16
Serine	1.04
Glutamic Acid	3.22
Proline	1.16
Glycine	1.77
Alanine	2.44
Cystine	0.00
Valine	1.61
Methionine	0.41
Isoleucine	1.04
Leucine	1.87
Tyrosine	0.78
Phenylalanine	1.05
Tryptophan	0.21
Total	22.46%

Table 119

ESSENTIAL AMINO ACID INDEX OF BIOLOGICAL VALUE OF ORGANIC MOLASSES PROTEIN

	Organic Molasses	Whole Egg	Egg Ratio	Log Egg Ratio
	Per Cent in Protein			
Lysine	7.00	6.98	100%	2.0000
Histidine	2.18	2.43	89%	1.9494
Arginine	3.82	6.10	62%	1.7924
Threonine	5.10	5.12	99%	1.9956
Cystine +	0.44	2.43		
Methionine	1.82	3.42		
	2.26	5.85	38%	1.5798
	7.16	6.85	100%	2.0000
Isoleucine	4.63	6.29	73%	1.8633
Leucine	8.33	9.17	89%	1.9494
Phenylalanine +	3.47	5.70		
Tyrosine	4.67	4.16		
	8.14	9.86	82%	1.9138
Tryptophan	0.93	1.29	73%	1.8633
				18.907

77.7 = 1.8907

Section VI

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Section VII

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